NEW METHODS FOR IMPROVING SENSITIVITY IN TRACE ANALYSIS

A THESIS

Presented to

The Faculty of the Division of Graduate Studies

by

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In Partial Fulfillment

of the Requirements for the Degree

Doctor of Philosophy

in the School of Chemistry

Georgia Institute of Technology

February, 1978

NEW METHODS FOR IMPROVING SENSITIVITY IN TRACE ANALYSIS

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Quota pars operis tanti nobis committitur?

ACKNOWLEDGEMENTS

I would like to express my gratitude to Dr. H.A. Flaschka for his aid and counsel in the conduct of this work and in the preparation of the manuscript. Dr. P.E. Sturrock, Dr. R.F. Browner, and Dr. P.B. Sherry also aided in the preparation of the manuscript. Mr. G.E. O'Brien aided in the design and construction of the instruments and circuits used in this work.

Dr. J.A. Bertrand, Director of the School of Chemistry, aided in the procural of an assistantship and also provided much helpful advice.

No list of acknowledgements would be complete without the inclusion of my co-workers: Mr. J.J. Tice, IV, provided a great deal of advice and support; Mr. C.L. Dobbs gave much needed information about the behavior of o-tolidine; Mr. R.D. Clemensen aided in the development of many of the computer programs used during the course of this work. For all this advice and other support too lengthy to list, I wish to express my appreciation to these people.

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SUMMARY

Several approaches for increasing the sensitivity of photometric determinations are explored. These approaches are based on increasing the magnitude of the parameters: absorptivity, a, effective path length, b, and effective concentration, c, of Lambert-Beer's Law, $A = abc$. Each of the approaches has provided increased sensitivity and it is felt that the techniques developed can be successfully applied to practical analysis when determining lower concentrations or using smaller samples or both.

Determination of Manganese

A chromogenic agent with a much higher molar absorptivity than that of permanganate was used in conjunction with a long path microcell to determine manganese at the ng/ml level. A selective oxidation of manganese in alkaline medium in the presence of triethanolamine followed by the addition of pyrophosphate and acidification of the solution is used to place manganese into the tervalent state. Manganese(III) is then allowed to react with o-tolidine to form an intensely colored quinonediimine. The absorbance of the quinonediimine is measured at 440 nm. The technique was successfully applied to the analysis of sea water.

Photometric Titration of Iodine After Chemical Amplification

The chemical amplification method first developed by Spitzy and co-workers was modified in such a way as to afford a more sensitive technique. A novel optoelectronic photometer was used in the actual titration. The procedure was found to work well at the μg/ml level of iodine.

Effect of Nonmonochromatic Light on Analytical Photometry

The effect of nonmonochromacy inherent in most light sources used in analytical photometry was studied. Calculations were carried out for both parabolic and gaussian curves. The deviation from the ideal situation, called the relative error, was calculated. It became apparent that with the use of chromogenes with higher molar absorptivities and longer path length cells, the working range in which a linear calibration curve can be expected is reduced.

Nomenclature

An attempt was made to clarify the issue concerning the misuse of many terms in analytical chemistry.

CHAPTER I

PRESENT STATE OF ANALYTICAL CHEMISTRY

Introduction

The quantitative determination of very small absolute amounts and very low concentrations of many species is one of the most important tasks of the modern analytical chemist (1). It is sometimes necessary to determine microgram and even nanogram quantities of chemical species in such diverse media as foodstuffs, drugs, industrial effluents, natural waters, and biological speciments, to name but a few. These highly different systems require the application of many methods to be extended into areas where the conditions are very different from those for which the methods were initially developed and applied. The demands placed on analytical methods have resulted in many notable advances. New methods have been developed and existing methods modified and refined to meet the new challenges.

Scientists have become increasingly aware that the presence or absence of very small amounts of a chemical species can have a profound effect upon the behavior of chemical, physical, and biological systems (2). A growing concern on the part of the general public about the effects of chemical additives and pollutants has given an impetus to analysts to develop methods capable of detecting and quantifying such species. Certain elements are now known to govern biological processes

at the cellular level. Medical researchers have linked many disorders with the amounts of certain species present in the body. Even drug addiction has been linked to certain chemical species (3). Industrial safety regulations are written with allowable limits of the least detectable quantities. Legal questions have also made it incumbent upon the analyst to be more certain of the validity of the results of the determination than ever before.

The problem of the determination of a material present in very small amounts and often at very low concentrations has become of major interest to analytical chemists mostly during the last two or three decades. The initial developments were in the field of microanalysis, that is, analyses that had definite constraints upon the sample size. Only a few milligrams or a fraction of a milliliter of sample was available to the analysts and the methods used had to be modified to work within these limitations. The first microchemists were involved in clinical studies and their methods were for the most part refined versions of existing standard procedures. One of the truly ingenious adaptations developed by analytical microchemists was the use of chemical amplification reactions as an analytical method.

The analysis of high purity materials, required by the electronics and metals industries, presented different problems. The sample size was usually not a limiting factor, but contamination of the sample preparation during the analysis proved to be a serious problem. The determination of entities at low concentrations is conventionally referred to as "trace analysis". The concept of trace analysis varied

in the course of time; the definition depending on the requirements of the analyst in the determination and on the actual state of development of analytical chemistry. The degree of difficulty presented is not only due to the need to determine a trace constituent, but also due to the fact that the determination must be carried out in the presence of high quantities of other species. The chemical environment in which the sought for species is present is usually termed the "sample matrix". Any factors introduced into the determination by the matrix are called "matrix effects". The sample matrix can present problems in the determination and in such a case would be termed a "hostile matrix".

Classical methods dominated chemical analysis through the first thirty years of this century. These methods are based entirely on the stoichiometric reaction of the sought for species with some reagent. Classical methods are also known as chemical methods and since these methods usually involve sample dissolution, they are also known as wet methods. An analysis involving these methods is often called a wet analysis. Classical, or wet methods encompass gravimetry, titrimetry, and gasometry.

The objectives of these classical methods were to operate on the sample in such a way that a chemical reaction took place, unique to the components that were to be determined. The analyst would weigh precipitates and residues, measure consumed or evolved gases, or titrate acid-base and redox systems. The main problem was to discover the reactions and reagents that lent themselves to the making of quantitative determinations and to refine and simplify the measuring techniques.

Very ingenious, reliable, and accurate methods resulted. In all cases, however, it was necessary to permanently change the sample substrate. In some cases the destructive nature of the methods was a problem.

Classical methods were extended to their limits of applicability in the endeavor to conduct trace analyses. Cyclic amplification procedures were developed, but even these methods had practical limits which were soon reached. Problems with sample size, interferences, and the complexity of the analytical methods forced the development of different approaches to the problem. Industry required less complex, faster methods of analysis than the existing classical ones when applied to trace analysis. While the analytical chemists tried to extend their classical techniques to suit the problem, developments in physics and physical chemistry provided some of the methods that industry desired.

Advances in technology allowed the analytical application of many techniques based on theories that had been in existence for a number of years. These methods are the ones that involve no chemical reaction in the actual determination and are termed physical methods. These physical procedures, in contrast to chemical ones, are largely concerned with energy changes. Physical methods are also known as instrumental methods because they require the use of instruments other than the balance and buret. The most important physical techniques are those based on the absorption and emission of radiation.

The first instrumental method to find broad application in industry was emission spectroscopy. It offered the industrial analyst a fast method for multi-element analysis, sometimes even at the trace

level. Emission spectroscopic techniques are still a major tool of the industrial analyst. Joining this rather simple method are much more complex procedures based on matter-energy relationships throughout the entire spectrum; x-ray fluorescence, neutron activation, radiometric, mass spectrometric as well as the more common absorption-emission methods all play important roles in trace analysis today. These methods could more correctly be termed dry instrumental procedures, since usually no chemical operations whatsoever are performed upon the sample. Electroanalytical methods, such as polarography or coulometry, do involve wet chemical operations, but usually only as an initial step in the procedure. Such methods can best be described as wet instrumental methods. All instrumental methods of analysis have undergone a period of tremendous development and found wide application in the last three decades. It is now possible to conduct analyses which were previously difficult, if not impossible, to complete. These instrumental analyses can be carried out rapidly by personnel possessing a minimal amount of formal training. Dry physical methods were viewed as a panacea for the ills of analysis by many chemists, but eventually the limits of these methods also became apparent. Some of the limitations were caused by inadequacies of the equipment available; advances in electronics have given new life in many cases as, e.g., thermal conductivity methods.

Most physical methods require for calibration a series of standards with a gross composition similar to that of the sample. The instruments themselves are often rather expensive and difficult to maintain at peak operating efficiency. The levels defined as trace also

decrease with the advance of time, surpassing the limits of purely physical methods. It became readily apparent that some new approaches would be necessary to solve the problem.

Fortunately, work on classical methods had not been fully abandoned, even though many scientists thought these methods to be passé. When the limits of many of the purely physical methods were reached, a merging of wet classical and dry instrumental methods began to occur. Various chemical pretreatments of the sample involved the operations of classical analysis and rendered the sample preparation more suitable for the physical finish intended.

The wet operations which were applied to the sample and the sample preparation enabled the analyst to achieve an increase in the concentration of the sought for substance in the sample preparation and to isolate the sought for component from many other components which might interfere with the instrumental method. The problem of providing the reagents and discovering the reactions and methods necessary to achieve the concentration increase (i.e., the enrichment) or to obtain the separation, is in the territory of the wet analytical chemist. Those analyses which employ traditional wet methods in combination with physical methods are termed physiocochemical methods. A better term, more indicative of the sequence of the operations, would be chemico-physical methods. This more clearly represents the fact that chemical treatments are performed upon the sample and the resulting sample preparation is subjected to a physical finish.

A chemicophysical analysis consists essentially of the study of the relationship between the composition and the properties of a system at chemical equilibrium. It is also common to speak of a chemicophysical method of separation, such as extraction or chromatography. Chemicophysical methods of analysis dominate contemporary analytical chemistry. One of the most common chemicophysical methods used in routine analysis today is photometry, either directly as a determination or indirectly as a photometric titration. The instrumentation required is relatively simple and inexpensive and many species can be determined.

The advent of chemicophysical methods may be hailed to be the final answer to the many problems encountered in trace analysis. Unfortunately this is not quite justified. With the ability to detect smaller amounts and lower concentrations questions arose about sources of loss and contamination within each determination. Many procedures involved multiple wet operations, each of which could introduce an error into the analysis. Since the species to be determined are present in such small absolute amounts and low concentrations, any loss or contamination error can be and often is enough to make the results spurious. While the loss of a milligram of the sought for species may be an entirely acceptable occurrence in an analysis where a gram of that species is present, the same milligram amount may be and often is many times more than the amount of the sought for substance present in the sample in a trace analysis. Although the limits of trace analysis have been pushed far beyond what was thought possible only ten or twenty years ago, the worth of the results of these analyses has become subject

to close scrutiny. Reagent purity, the conditions of the analysis, the number of manipulations, the equipment used; all of these factors are now recognized as affecting the validity of an analysis.

Chemists recognize the need to maintain or improve the practical quality of the methods of analysis. It is necessary to minimize the number of operations involved in the method, since each operation may be the source of an error. The purity of the reagents necessary for a method was further defined to set specific allowable limits on certain impurities. No longer is a "spectrally analyzed" grade solvent always pure enough. Manufacturers now offer "ultrapure" solvents and reagents for use in trace analysis. Where possible, the steps of enrichment and separation are usually combined into a single manipulation, typically a solvent extraction.

This is the present state of chemical trace analysis. Analysts are attempting to improve the quality of the analyses while simultaneously increasing the speed and applicability of the methods in use. The present trend is to regress to the classical wet methods and attempt to adapt these procedures to allow an instrumental finish. The major thrust is towards the more easily conducted photometric finish.

Nomenclatural Remarks

With the growth of analytical chemistry and the eventual combination of many classical and physical techniques, many terms that previously had well defined meanings became ambiguous. One of these, for example, is "trace". While definitions exist for trace and the often used

prefixes "micro", "ultra", etc., they are interpreted differently by different people. The general definitions put forward by international institutions such as IUPAC have not been completely accepted by the analytical community. Even the rather basic terms "quantitative analysis" and "determination" are confused by some and taken as synonomous. Analysis refers to the sample material as the object of activity, as, e.g., the general statement "limestone is analyzed" or more specifically, "limestone is analyzed for its nickel content". Determination refers to the sought for species, or analyte, as the object of activity, as, e.g., "nickel is determined in limestone". Analyte is also another example. The term correctly refers to the sought for species. Many analysts wrongly use analyte to refer to the sample solution.

A particularly bad situation exists with regard to descriptions of the quality of a detection and determination. The relevant terms have taken on different meanings because of the separate development of the two main branches of analytical chemistry, namely chemical and physical. References to the sensitivity, selectivity, and specificity of techniques, methods, or reagents occur frequently. The definitions that serve the wet analyst and the physical analyst separately do not perform as well for the chemicophysical analyst. It is therefore important to give the definitions as they will be used in the present work and to add some remarks where appropriate.

Reagent, Reaction, and Test

There is little ambiguity associated with these terms when they are used in general chemistry, but confusion arises when they are used

in conjunction with analytical methods. Their meanings in wet qualitative analysis were clearly defined by Feigl. Although certain modifications were necessary in the change from qualitative to quantitative analysis, the meanings are essentially the same. The major alteration is that the objective of the method is now the determination, rather than the detection, of a sought for species.

The first member considered was the reagent; it is defined as a chemical that participates in the detection. The solvent is not included because it merely provides the medium. One may qualify a reagent as specific, that is, reacting with only one species, or as selective, that is, reacting with two or more species. These definitions will suffice for now, but a more detailed discussion will follow in the next sections. It is best to develop the topic in conjunction with an example, namely dimethylglyoxime, DMG. One may encounter the phrase "DMG is a reagent specific for nickel". This is incorrect. As a reagent per se, DMG is not specific, or even reasonably selective, as it reacts with about twenty metals. However, with only two metals, namely palladium and nickel, does DMG react to give a precipitate. With all the others the reaction leads to a soluble complex. Now, considering DMG as a precipitating agent, it suddenly appears highly selective. Thus, it is more advantageous from the point of view of the analyst to speak not of the reagent but to refer instead to the reaction. Then it can be stated that DMG participates in two highly selective reactions, namely the precipitations of nickel and palladium. Taking a closer

look, another fact comes to light. Palladium precipitates in acidic medium where nickel does not. Nickel precipitates in ammoniacal medium where palladium remains in solution. Thus, DMG, an unselective reagent, can now be made to react rather specifically in these two cases. Obviously which reaction occurs is dependent on the conditions. So, when the milieu is taken into consideration, one may speak about the "test", which includes the reagent, the reaction and the environment. It is apparent that it is quite important to distinguish between the terms reagent, reaction, and test. Unfortunately, the latter two are often used synonomously and confusion results.

The preceeding discussion was developed from a qualitative analytical chemistry viewpoint; that is, for detections. Analogous reasoning can be made for quantitative analysis; that is, for determinations, where the conditions are somewhat more critical and need more careful control.

That a test or determination is specific or selective should not, however, imply that it will work under all conditions. Interferences can and do occur.

The concept of an interference needs explanation. There is, unfortunately, a dichotomy associated with it. The correct definition of an interference is any substance whose presence causes a test or determination to function improperly or which makes the interpretation of the result difficult. Many chemists also refer to the phenomenon itself as an interference. The interchange of cause and effect creates confusion. Therefore, the former definition will be used exclusively in

this work. For purposes of discussion, interferences will be considered as being members of one of three groups. Firstly, there are those substances which give a signal of the same type as that of the analyte. These species are commonly called positive interferences. Secondly, there are those substances whose presence causes a reduction in the analyte's signal, perhaps to the point of extinction. Lastly, there are those substances that do not effect the analyte's signal, but make its recognition difficult or even impossible.

Substances that are members of the first group can be the most troubling. As an example, consider the photometric determination of manganese by measurement of the purple permanganate species. The reagents used for the oxidation of manganese to permanganate will also oxidize chromium to dichromate. Although the two colors are visually easy to distinguish, the photometer will not be able to tell the difference between the two species at the measurement wavelength. Therefore, the resulting signal will be a sum of the signal from the permanganate and the dichromate and will indicate a higher manganese concentration that really exists. A similar situation occurs in the gravimetric determination of chloride as silver chloride. If any bromide or iodide ions are present, they will also precipitate upon the addition of the silver nitrate reagent. Species that are members of this interference group always cause the results of a determination to be higher than the true value, hence they are called "positive interferences".

Members of the second group listed are substances whose presence prevent the completion of the analytical reaction. Two examples can be cited in the case of the gravimetric determination of nickel by precipitation with DMG. If any cobalt is present, it will complex with the DMG and may not leave enough of the reagent free to completely precipitate the nickel. If any cyanide ions are present, they will form a soluble complex with the nickel, and thereby prevent its reaction with the DMG.

In the flame photometric determination of calcium, any refractory material present in the sample preparation can prevent the complete excitation of the calcium in the flame. In all these cases, the analyte's signal will be lessened, leading the analyst to conclude that there is less of the sought for species present that there really is; such substances are commonly called "negative interferences".

Members of the third group include the sample matrix and properties of the sample preparation. These interfering substances do not enter into any of the reactions of interest, but rather obscure the results. A visual titration into a highly colored medium is an example. The indicator used will change color as the titration proceeds in a normal manner, but the color change will be "screened" by the intense color of the sample preparation. An analogous situation occurs in the polarographic determination of cadmium in the presence of lead. The half wave potentials of the two metals are sufficiently separated for the determination to be carried out when both are present in approximately equal low concentrations. If, however, the lead is present in a much higher concentration than the cadmium, the lead wave will dominate the

polarogram and the wave from the cadmium will be submerged in it. The cadmium wave is effectively screened by the lead wave.

Interferences effect neither the specificity nor the selectivity of a determination, but rather restrict the number of situations in which a given method can be successfully applied.

Specificity

The International Commission on New Analytical Reactions and Reagents (ICNARR) has defined as specific those reagents or reactions which, in the test, are totally unambiguous for one species. The previously mentioned use of DMG in ammoniacal solution for the determination of Ni illustrates this idea. The concept of specificity can be extended to the dry physical techniques.

A specific method is not necessarily restricted to one single entity for detection and determination. The method may allow the detection and determination of many species, but each species can be treated independently of all the others. Emission spectrography can be cited as an example. Many elements can be detected and determined in a single film exposure provided there are not too many interferences. In accord with ICNARR terminology, this present work will define specificity as that characteristic of a method that allows the unambiguous independent determination of a species.

Selectivity

ICNARR has adopted the following definition for selectivity: that property of reagents, reactions, or tests that makes them characteristic for a limited number of species. This definition is in accord

with that used by the classical analyst. In contrast to the absolutism of specificity, there exist degrees of selectivity. The fewer the species that correctly cause a positive result, the greater the selectivity of the test. Many indices and systems have been proposed for the classification of selectivity, with some of the more reasonable put forward by Belcher and Betteridge (4), Cheng (5), and Wilson (6). These various indices indicate the conditions of the tests and determinations, and interferences which might exist. In reality, not only the selectivity is considered, but rather the entire test or determination. Therefore, such indices are more a description of the overall usefulness of the method than merely a measure of the selectivity.

The instrumentalist, on the other hand, considers selectivity as a measure of the extent to which an instrument is capable of differentiating between the signal due to the sought for entity and any other signals. The instrumental selectivity can depend to a great extent on the quality of the equipment involved in the measurement.

In this work, both types of selectivity will be used. The chemical selectivity in connection with the wet operations and the instrumental selectivity in connection with the dry finish. It is difficult to define an overall selectivity of a chemicophysical method, so this parameter will always be considered in its component aspects. With continuing improvements in instrumentation, the chemical selectivity is often the dominant factor.

Sensitivity

No other parameter can give a better impression of the applicability of a method than its sensitivity, but discussions of this term have always posed problems. Even qualitative analysts had problems with defining the meaning of sensitivity. In his discussion of methods for qualitative analysis, Feigl was the first to put forward a clear definition of sensitivity. He defined it as that property of a chemical reaction that allows a "practical, rapid detection of the smallest possible quantities at the highest possible dilutions". This definition combines the concepts of amount and concentration. Realizing that at times it might be convenient to speak of these separately, Feigl proposed the use of two terms, the identification limit and the sensitivity limit. In time, the names associated with these concepts have been somewhat changed.

The identification limit has become known as the limit of detection. It is the minimum quantity of a material that can be detected by any method, regardless of the volume. The sensitivity limit is known as the limit of concentration, but more commonly its reciprocal is used, namely the limit of dilution. The limit of dilution is the ratio of the quantity of solvent to the unit weight of material detected. In wet analysis, the limit of detection is a statement of the quantity sensitivity, while the limit of dilution gives information about the actual applicability of the method. These terms, collectively known as the chemical sensitivity, will be used in this work to describe the quality of the chemical methods employed.

Unfortunately, the working definitions of sensitivity developed by classical analysts are not the same as those of the instrumentalists. This is due to the previously mentioned historically independent development of the two analytical branches, with most of the initial instrumental definitions derived from spectrography. The instrumentalist defines sensitivity as the ratio of the magnitude of the changes in the response of an instrument to the magnitude of the changes in the quantity measured. Realistically, sensitivity denotes the ability of the technique to detect small changes in amounts of a substance. When the desired physical property is measured, the results are usually presented graphically in what is commonly called a calibration curve. For a photometric determination the plot is of absorbance versus concentration. An example is shown in Figure 1. It is obvious from the definition of instrumental sensitivity given above that it is desirable to have a curve with a steep slope, or more realistically, to make the necessary measurements on the steepest portion of the curve, so that very small changes in concentration will result in a large change in absorbance. Because of this relationship of the sensitivity to the slope, this characteristic has been called the slope sensitivity.* Superficially, it would seem that a high slope sensitivity would ensure a low limit of detection. This is not necessarily so. The operating characteristics of the photometer have a very important effect upon the ultimate sensitivity of the determination. Every signal output by the

*See Mandel and Stieler, J. Res. Nat. Bur. Stds., Vol. 53 (1954) 155-159 for an excellent mathematical formulation of this concept.

photometer has a certain, hopefully small, amount of variability associated with it. This variability, which is random and irreproducible, is more commonly called noise. Noise is present in the 0%T signal, the 100%T signal, as well as any signal output by the sample preparation. As one regresses along the curve in Figure 1, the value of the absorbance signal decreases. The value of the noise signal remains essentially constant. In Figure 2, the lower line represents the noise signal. The points labeled 1, 2, 3, etc., are the values of the absorbance signals read from the similarly labeled points in Figure 1. As one observes, the points corresponding to the absorbance values read from the steepest portion of the calibration curve lie very near and even below the line representing the noise signal. A criterion of an analytical signal magnitude of at least the sum of the noise and three times its standard deviation has been established for an instrumental measurement to be considered valid. The upper line in Figure 2 represents this minimum necessary analytical signal. This requirement provides an instrumental limit of detection analogous to the one developed by Feigl. This instrumental limit of detection will be called the basic sensitivity. It represents the smallest quantity or lowest concentration of a species that must be present to produce an analytical signal that meets the previously outlined criterion.

These are the two terms which will be known collectively as the instrumental sensitivity. They were developed with regard to their counterparts in the chemical sensitivity term. The limit of detection, or basic sensitivity, and the slope sensitivity will be used in this work to refer to this property of an instrumental technique.

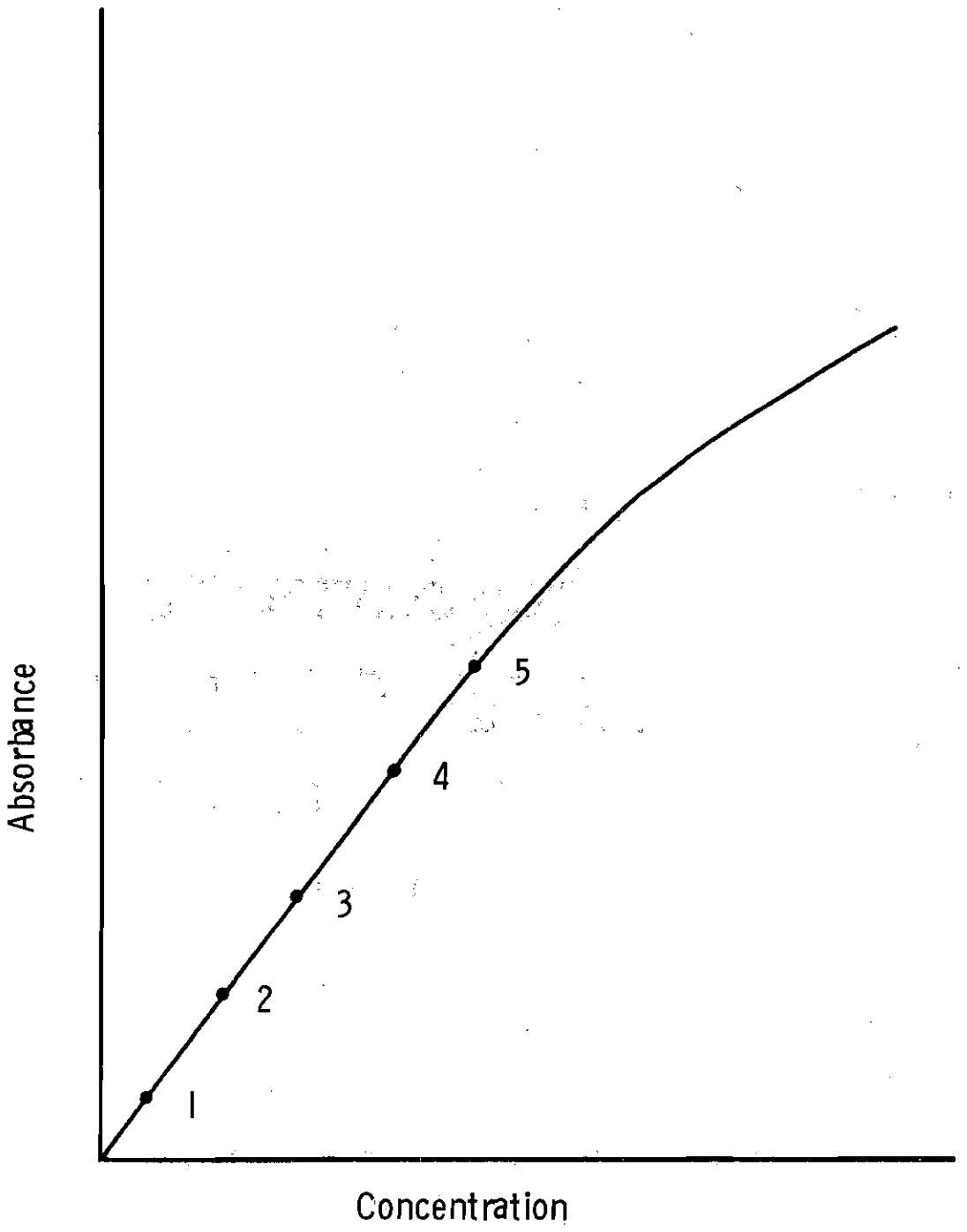


Figure 1. Calibration Curve for a Photometric Determination

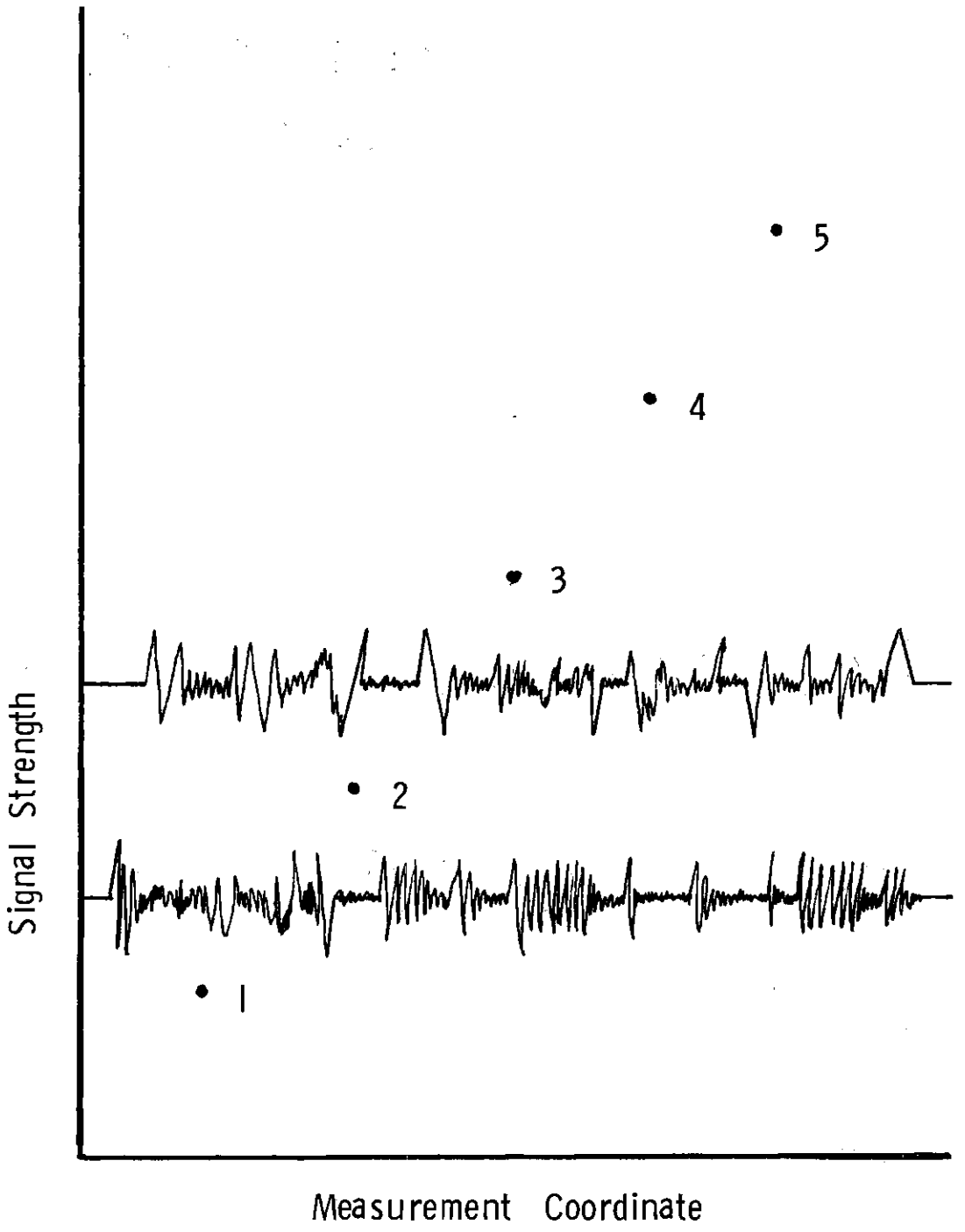


Figure 2. Illustration of Instrumental Limit of Detection

The chemical and instrumental sensitivities together compose the overall sensitivity. There is no agreed upon mathematical way of combining these component terms to arrive at the overall sensitivity. While it would be desirable to describe the method in terms of its overall sensitivity, there is no agreed upon definition to serve as a guideline, so this work will always refer to the chemical and instrumental sensitivities separately.

Technical Quality

It is necessary to consider the practical worth of a method before any judgment can be made as to its actual applicability. This practical worth should be arrived at by considering not only the overall sensitivity, but also several other factors. Among these are the reproducibility of the method, the time required for it, the training necessary to conduct it, and the facility with which it can be carried out. Reichel introduced the concept of "technische Güte" (i.e., technical quality) to represent this concept. Wilson (6) chose to term the various components as performance characteristics, as did Pszonicki (7). The proposals of these various scientists all try to define sensitivity, selectivity, and specificity in mathematical formulae. Such definitions tend to take away from the "feeling" the analyst develops for a method, and therefore must be taken very carefully if one is basing the evaluation of a particular method upon them.

It is quite evident that if there is an "equal among equals" in the technical quality of a method, it is probably the overall sensitivity. Therefore, to make a technique better for use in trace and micro

analysis, which is the thought here, it is necessary to improve the overall sensitivity, but not at the expense of the technical quality. In this present investigation, two spectrophotometric methods, namely a determination and a titration, are studied. In each case, both the chemical and instrumental sensitivities are dealt with. Chemical amplification reactions, selective redox reactions, and novel spectrophotometers were utilized.

CHAPTER II

THE PHILOSOPHY OF INCREASED SENSITIVITY IN PHOTOMETRIC ANALYSIS

Application of Photometric Methods

The color of a solution has long been utilized to make judgments about the concentration of a dissolved substance. Quite early, men in the Fertile Crescent were appraising the possible watering of wine by observing the color. Colorimetry, the predecessor of absorption spectrophotometry, may therefore be considered as one of the oldest quantitative analytical methods. Vierordt (8) is generally acknowledged as the one who advanced quantitation by being the first to produce a spectrophotometer and apply it to analytical chemistry. At the present time, photometric techniques comprise the greatest portion of chemicophysical analytical procedures.

One need only consider the broad applicability of photometers and spectrophotometers to understand their wide usage in both organic and inorganic analysis. With photometry it is not only possible to detect, identify, and determine dissolved species but also suspended or particulate matter. Photometric methods are also generally endowed with high sensitivity, while the necessary instruments are comparably inexpensive and easy to operate.

The fact that many substances can be determined spectrophotometrically indicates the technique's weakness. Spectrophotometric procedures

are generally unselective. Therefore, unless appropriate measures are taken, the probability of encountering interferences is high. Some degree of isolation of the sought for species is often required. Separations, while sometimes difficult or even impossible, are available for a large number of applications. In the many systems in which separations can be achieved, the spectrophotometric measurement itself usually is a simple task. The ability to determine trace quantities of a substance in an easy and rapid manner is one of the primary advantages of photometric analysis.

Requirements of Photometric Techniques

The most important requirements for satisfying the demands of modern analytical chemistry are selectivity and sensitivity. Once the primary requirements are met, attention is directed towards the secondary needs, namely, speed, facility, and ruggedness of a method.

Historically, photometry has afforded one of the best realizations of all of these qualities. Some of the most entrenched methods require nothing more than simply dissolving the sample material, perhaps adding a chromogenic reagent, and concluding the determination by making the photometric measurement. In more complicated cases, however, and especially when applied to trace analysis, extensive manipulation of the sample solution may be necessary to obtain an absorbance reading at a useful level. Some of the approaches required to achieve an absorbance increase, or as one may say simply (but not fully correctly), sensitivity increase, will be discussed in the following sections.

Increasing the Sensitivity in Photometry

As was pointed out in the discussion of the concept of sensitivity, it is desirable to have a high signal, in this case a high absorbance reading, for the concentration prevailing. As colorimetry evolved into photometry, and the instruments used became more complex, it became possible to amplify the signal output by various electronic techniques. As photometry progressed into the detection and determination of increasingly lower amounts at lower concentrations, the signal became close to, and even fell below, the noise level. When this occurred, simple amplification became ineffective, since both the signal and its accompanying noise were amplified, and the critical parameter, the signal to noise ratio, was left unimproved. Therefore, the discussion here will not deal with output amplification but will consider ways of obtaining a higher absorbance reading by operation with the parameters of the Lambert-Beer Law.

The Lambert-Beer Law states the linear relationship between the absorption of a beam of monochromatic light passing through a solution and the three parameters relating the instrumental setting and/or the concentration of the absorbing species as

$$A = abc$$

where

A = the absorbance

a = the absorptivity, which is a measure of the ability of a species to absorb light of a certain wavelength

b = the length of the light path through the absorbing medium

c = the concentration of the absorbing species

The possibilities for obtaining a higher absorbance reading can be recognized by examining the law.

Increasing the Absorptivity

If one considers fixed values of b and c, it is apparent that any increase in the absorptivity, a, would result in a higher absorbance reading. Some modest increases have resulted from merely altering the milieu. Flaschka and Barnes (9) and Flaschka and Tice (10) have used this approach in the determination of cobalt and silica, respectively. By adding sulfolane to the sample preparation the absorptivity was approximately doubled. While this may be sufficient to allow a previously borderline determination to be achieved, it is certainly not spectacular.

In further consideration of the problem, certain facts must be taken into consideration. While many organic substances exhibit exceptionally high molar absorptivities, especially in the UV regions, most inorganic species have rather low absorptivities. It is often necessary to convert the inorganic species to an equivalent amount of a more highly colored entity, as classically e.g. manganese to permanganate, or copper(II) to copper(II) tetrammine. The limits of such classical means have rapidly been reached. Greater enhancements are needed. The approach that is presently most often employed is the reaction of the analyte with an appropriate agent to produce a highly absorbing entity.

Most such agents are organic compounds. Braude (11) has shown that the highest theoretical value of molar absorptivity is of the order of 100,000 liters per mole-cm. Common values of the molar absorptivities of the species employed in the most sensitive procedures lie in the range of 20,000 to 40,000. Thus a two- to five-fold gain is all that can be hoped for in these methods when considering a change in a. But for those species with molar absorptivities in the range of 10,000 to 20,000 (of which there are many), significant gains will result. One must, of course, realize that when switching to another reagent, either an already existing one or a newly synthesized one, a complete reevaluation of the analytical situation becomes necessary. Milieu effects, interferences, temperature effects, etc., all have to be studied. In other words, a new procedure must be developed.

Increasing the Path Length

Obviously, increasing the path length brings about an increase in the absorbance reading. The most common path length for a spectrophotometric cell is 1 cm, and cells with 5- or 10-cm path lengths are available. Such commercial cells pay for the increased path length with a usually more than proportional increase in the volume of the liquid required for filling. Such a volume increase is undesirable and actually detrimental to any efforts made for enrichment prior to the photometric finish. In order to keep the volume low, the logical step would be to employ a microcell. What is commonly understood as a microcell, however, is a cell that is filled with very small amounts of

liquid, but with a path length of millimeters or even fractions thereof. This is, of course, unsatisfactory for the already low absorbances encountered with the solutions under discussion.

Increasing the Effective Concentration

Operations upon c in order to increase the absorbance reading may at first glance seem absurd. After all, the photometric measurement is made to establish this quantity, so that a conclusion can be drawn as to the composition of the sample material. Therefore, some additional comments on this point are necessary.

The final goal of the analysis is to determine the amount of sought for substance in a given amount of the sample material. The amount present in the sample taken is derived from the concentration, c, measured photometrically, and the volume of the sample preparation for which this c is measured. Once this is accomplished, the content of the sought for substance in the original sample material can be obtained through simple calculations. While the amount of sought for substance brought into the process is fixed by sample weight and content in the sample material, the effective concentration, c, in the final sample preparation, is subject to manipulation. The sample is processed in such a way that the final volume in which that amount of sought for substance is present is as small as possible. Such processing is often called preconcentration or enrichment.

There is, of course, the trivial approach of increasing the size of the sample used for the analysis. This is not always possible. Even

when the analyst can exercise control over the taking of the sample, there is often a limited amount of material to work with. This is especially true in clinical analysis. Another problem may occur, namely that of too many interferences from an overly great amount of sample. Therefore, other ways to increase the effective concentration are preferred.

The effective concentration can be most conveniently increased through the use of an extraction. Extractions have been extensively employed for enrichment, but few deliberate attempts have been made to exhaust their potential. Extraction inefficiency and difficulty in recovering small volumes of extract have limited the enrichment. Enrichment extractions become exceedingly difficult with small or micro samples. Homogeneous extraction (12) and solid extraction (13) techniques have been developed to aid in the solution of the problem

An indirect way to increase the effective concentration is through the application of chemical amplification reactions. Such reactions and their use in trace analysis are discussed in Chapter V.

Combination of Approaches

While each of these approaches may independently increase the sensitivity of a given method, a combination of the possibilities should give a greater gain. A new reagent may yield a ten-fold increase in the resulting procedure's sensitivity; coupling this with a long path micro-cell makes a hundred-fold increase possible, at least theoretically. An increased effective concentration may give only a four- or five-fold

increase in sensitivity; if a long path cell is also used, a forty- or fifty-fold increase becomes possible. The ideal situation would be to develop a method in which all three parameters are at their maximal feasible values.

CHAPTER III

LONG PATH PHOTOMETRY

As was mentioned in Chapter II, a very simple way to increase the sensitivity of a photometric method is to increase the optical path through the cell. There are two approaches possible. One is to employ multiple internal reflections within the cell. This possibility was examined by Flaschka and Barnes (14) and found to have several difficulties. The other is to expand the cell length while keeping the diameter small and thereby also the volume.

The design and construction of long path microcells have been discussed in the literature (15,16). The two major problems encountered in their use are finding a photometer that will accommodate a 20-cm or longer microcell and ensuring proper and reproducible alignment of the cell in the light path.

Many commercial instruments will accept a cell of 5-cm, and some even accommodate one of 10-cm. It is, of course, possible to modify a conventional photometer so that it can accept cells of greater length. However, this is only readily possible with certain instruments, namely those that have the cell compartment as a separable unit between lamp housing and detector. Changes of this sort have been extensively discussed in conjunction with the development of phototitrators and a comprehensive listing can be found in the review by Leonard (17).

There is, however, a basic difference that should be considered. In a photometric titration the primary concern is not the reproducible alignment of the cell within the light path; it rather suffices to keep the position rigidly constant during a particular titration. In a photometric determination the alignment must also be fully and accurately reproducible, a difficult task when operating with long and narrow cells, where there is the additional problem of getting enough light into and through the cell. It would be advantageous to have perfectly collimated light to help achieve this, but, unfortunately, very few commercial instruments have a high degree of collimation within the sample cell compartment. The exceptions are laser photometers. Quite often, the optical geometry is such that a focal point exists somewhere near the middle of the cell housing. One can, of course, align the cell as well as possible and then fix it permanently in that position; this is at best a very poor solution of the problem, since it creates a single beam photometer. Most modern spectrophotometers are double beam instruments and the reduction to single beam configuration wastes some of the added features included in a double beam instrument. More seriously, the second beam is used to compensate for fluctuations in light intensity. This compensation would be lost and the instrument would not show the required stability. It seemed, therefore, better to expend the efforts on the design and construction of a photometer starting with existing and readily available components, rather than to concentrate on modifications. In line with this approach, Flaschka and Barnes (14) used the

monochromator and a few other parts of a Bausch and Lomb Spectronic 20 to build a long path photometer.

The next logical step in the development was to use a modular photometer assembled in such a way as to permit inclusion of a long path cell. Many companies have begun to produce modules that can be "mixed and matched" to build a photometer and thus provide possibilities for accommodating cells of just about any path length. A photometer assembled on this basis was used in a part of the present investigation and is described in Section I.

Another way of dealing with problems of alignment and light path came about with the availability of optoelectronic devices. Photodu-odiodes and the more recently developed and significantly more respon-sive phototransistors have a light sensitive area of about the same size as the diameter of the cell, so that such devices can be glued or otherwise permanently affixed to the cell exit window with no loss in the utilization of that area. In this way, half of the alignment problem is eliminated. As the next step, a light emitting diode (LED) can be affixed to the entrance window of the cell and the alignment made perfect and permanent. At the present time, the variety of available wavelengths is limited, thereby restricting the number of applications. However, with the interest in the LEDs, hope is justified that the situation will soon change.

Instruments built with optoelectronic devices as light source and detector are rugged, portable, inexpensive and require very low power. These characteristics make such instruments ideally suited for application

in the field (18). It is, of course, possible to make use of the advantages of the optoelectronic devices also in a more elaborate way. One instrument resulting from such efforts is described in Section II.

The general requirements of the various components of a spectrophotometer can be found in any general text on instrumental analysis (36). There are, however, certain additional aspects that must be considered with regard to the instruments required in the present investigation.

It must be realized that all of the components work together to function as a photometer. They must be chosen with regard to their compatability with one another. Once assembled into a photometer, a change of any one of them will most probably effect the others. If a more intense light source is used, the radiant energy exiting the cell may overwhelm the detector. If a better monochromating device is used, then the radiant energy reaching the detector may be insufficient. The instrument builder must always recognize the interdependence of the parts of a photometer and should chose the components to give in concert the best overall performance.

There are aspects that require special attention due to the peculiarities of the present investigation. Two problems merit preferred mention in this respect: monochromacy of the light and the difficulty of getting enough light into and through the microcell.

The theoretical derivation of the Lambert-Beer Law postulates strictly monochromatic light. In actual analytical practice, relaxation from this requirement is allowed. To the analyst, adherence to the law

commonly implies a "nice" calibration curve, that is, the plot of concentration versus absorbance, to be a straight line. Such a line will be obtained even with nonmonochromatic light when the absorbance readings are low, that is, commonly at low concentrations of the absorbing species. This is precisely the case in trace analysis where, as a consequence, monochromaticity requirements can be relaxed. A half width of 10 to 20 nm, or even more, as encountered in simple interference filters and LEDs, is fully adequate. It is of great importance to clearly point out here that with "nonmonochromaticity" reference is to be understood to a large band width; it does not imply that two or more greatly differing wavelengths are allowed. The latter case is to be dealt with under the term "stray light" and some consideration to this problem will be given in Chapter VII.

Most ideally, a laser would solve both of the problems mentioned above in the simplest manner. The light is of nearly perfect monochromaticity and collimation. The latter means that no difficulties arise in getting the narrow parallel beam into and through the small cell. Such light sources, although great progress can be noted in recent literature, are still cost-wise incommensureable with the intended simple approaches desired for the instruments.

Under the present circumstances, ideally, well collimated light would be the prime choice. Collimation, however, becomes a greater problem with the narrowing of the beam. Of course, there is the possibility of collimating a beam of large diameter and using only a small area of its cross section, preferably the center portion. This approach

would bring a well defined beam into and through the microcell but would necessitate a rather high intensity light source, since only a portion of the emitted radiant power is used. The loss of radiant power as such should not be of consequence, but the high intensity source presents another problem. Because with the given design a single beam instrument is involved, a highly stable light source is essential. The stabilization of the power is more involved and expensive the higher the power; consequently this approach is not the best.

The fact that one deals with a single beam instrument, however, alleviates the situation in a certain way. In such an instrument the light path must be fixed, but not necessarily in one particular way. Thus the prime consideration is to get the light into and through the cell in a fixed way, regardless of its geometry. It is therefore possible to collect the light in such a way that the beam has a focal point at the entrance window of the cell. Then all, or nearly all, the light will enter the cell. But when the angle of attendance is too obtuse much of the light will be refracted into the glass walls of the cell, never enter the solution again, and thus be lost for the absorbance measurement. The situation can be improved by using a lens of rather long focal length, but then the arrangement becomes susceptible to even minute alignment changes.

The lamp used as a light source is a polychromatic lamp, so monochromation is necessary. The monochromating device may be positioned either before or after the cell. Inserting it before the cell is the preferred way; if placed after the cell, the sample solution would be

exposed to the full energy of the polychromatic radiation, with the attendant problems of warming the solution and its consequences. When the monochromating device is placed before the cell, one must realize that as far as the light path is concerned the exit aperture of the monochromating device has to be considered the "source", and all the arguments presented in the preceeding discussion transfer now to it.

Similar alignment problems exist at the exit window of the cell. One can use an optoelectronic device, such as the photoduodiode or phototransistor, as a detector. Such devices exhibit a broad spectral response, and are simple, rugged, and inexpensive. Similarly to the LED, they can be permanently affixed to the cell window, thus solving alignment problems. However, there may be certain cases in which these devices are not suitable. The radiant energy reaching the detector may not be sufficient to allow the phototransistor to respond. In such cases, a photomultiplier tube is a better choice.

Photomultipliers are capable of relatively high current output (10^{-3} ampere) and are able to respond to very low levels of radiant energy. However, such devices require a more complex power supply than the optoelectronic detectors mentioned. A more conventional photometer, assembled from modules and incorporating a photomultiplier tube as a detector, was used in the photometric determination of manganese described in Chapter IV, while a photometer assembled using optoelectronic devices as light source and detector was used in the photometric titration of iodine described in Chapter VI.

The preceeding discussions have presented the basic requirements for photometers to be used in conjunction with the present investigation. Two principal approaches emerge; a conventional one, involving a polychromatic light source, monochromating device, cell, and photomultiplier tube, and a more novel one, involving solely optoelectronic devices and the cell. The instruments realized from these two approaches are described in the following sections, along with a long path microcell that could be used with either instrument.

Section I: Modular Photometer

Several companies make modules that can be used in the assembly of a photometer for long path work, but McKee-Pedersen also produced a rather unique coupling system that allows some novel applications of the modules. The male-female couplers produced by McKee-Pedersen allow the use of a cell of any feasible length with no problems in the reproducibility of alignment. A block diagram of the various modules in the arrangement used is shown in Figure 3.

The light source is a McKee-Pedersen model MP-1019 with a GE 1763 bulb operating from six volts, supplied by a McKee-Pedersen MP-1026 regulated power supply. The MP-1019 is fitted with a rotating shutter which allows rough control of the light output. The MP-1026 has the stability necessary for use in a single beam instrument, and it has the added advantage of permitting fine adjustment of light intensity by varying the voltage setting.

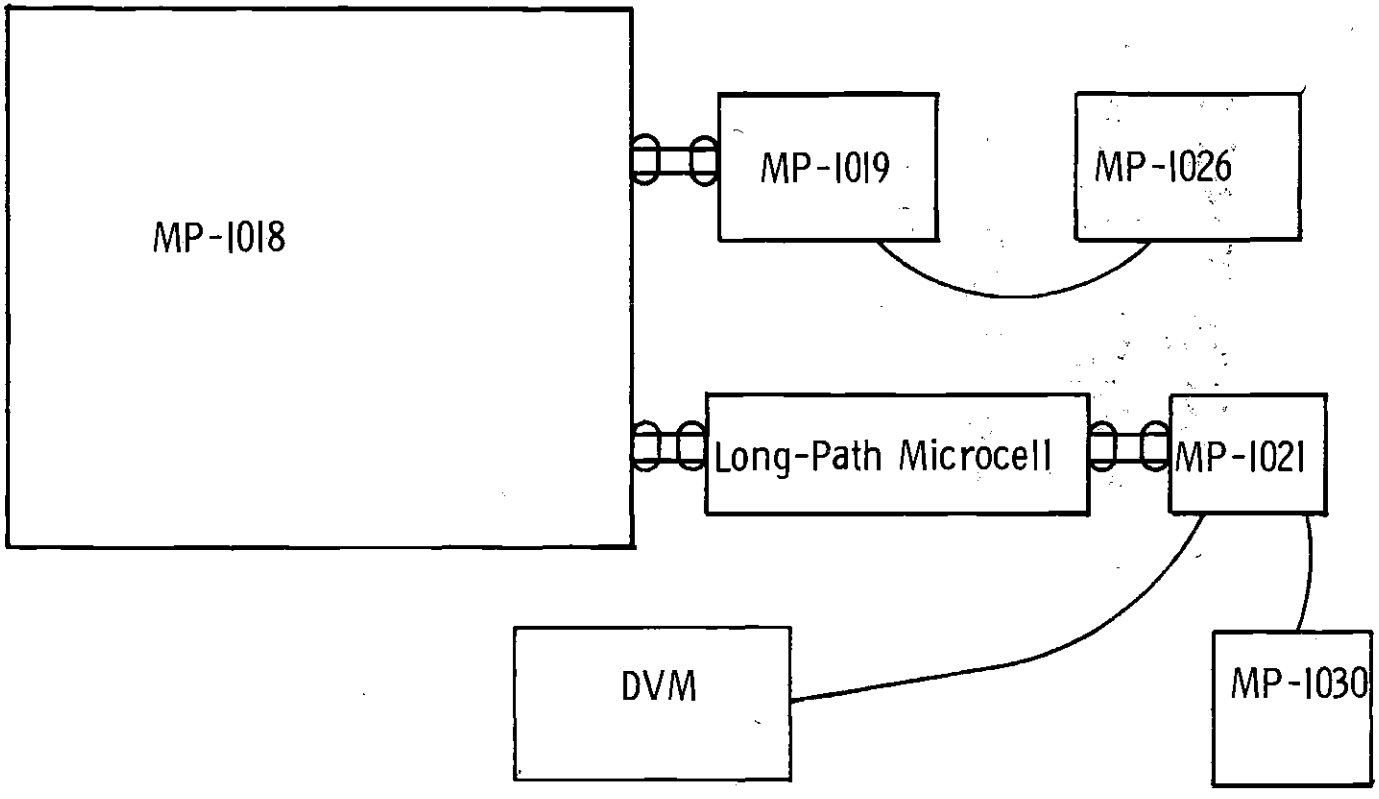


Figure 3. Modular Photometer

The monochromator is a McKee-Pedersen model MP-1018. It has a Czerny-Turner configuration and uses a 590 line/mm replica diffraction grating blazed for 400 nm. The wavelength is directly readable on the turns counter to ±0.2 nm. The MP-1018 is of far higher quality than is necessary for the present investigation, but it can be used in conjunction with other methods where a higher degree of monochromacy is required.

The long path microcell is described in section III.

The light detector is a McKee-Pedersen model MP-1021, equipped with a RCA 931A tube. The MP-1021 will accept any side-on nine stage photomultiplier tube. The RCA 931A was chosen because it has its maximum response in the spectral region of interest. The spectral response curve is shown in Figure 4.

The power supply for the photomultiplier is a McKee-Pedersen model MP-1030. This high voltage power supply can deliver 3.0 milliamps at 500 to 1000 volts and is equipped with a current limiter.

The male and female couplers mentioned earlier are McKee-Pedersen models MP-1891 and MP-1892, respectively.

The digital voltmeter used for the readout is a Kiethley Instrument model 160. It is a solid state, line operated multimeter with the convenience of a digital display. The photomultiplier voltage is read as percent transmittance and then converted to absorbance.

A modular photometer, such as the one described above, is welcomed for research and general application because of its versatility. The unique coupling system enhances the versatility even further. It is possible to machine a plug, containing either a LED or a phototransistor,

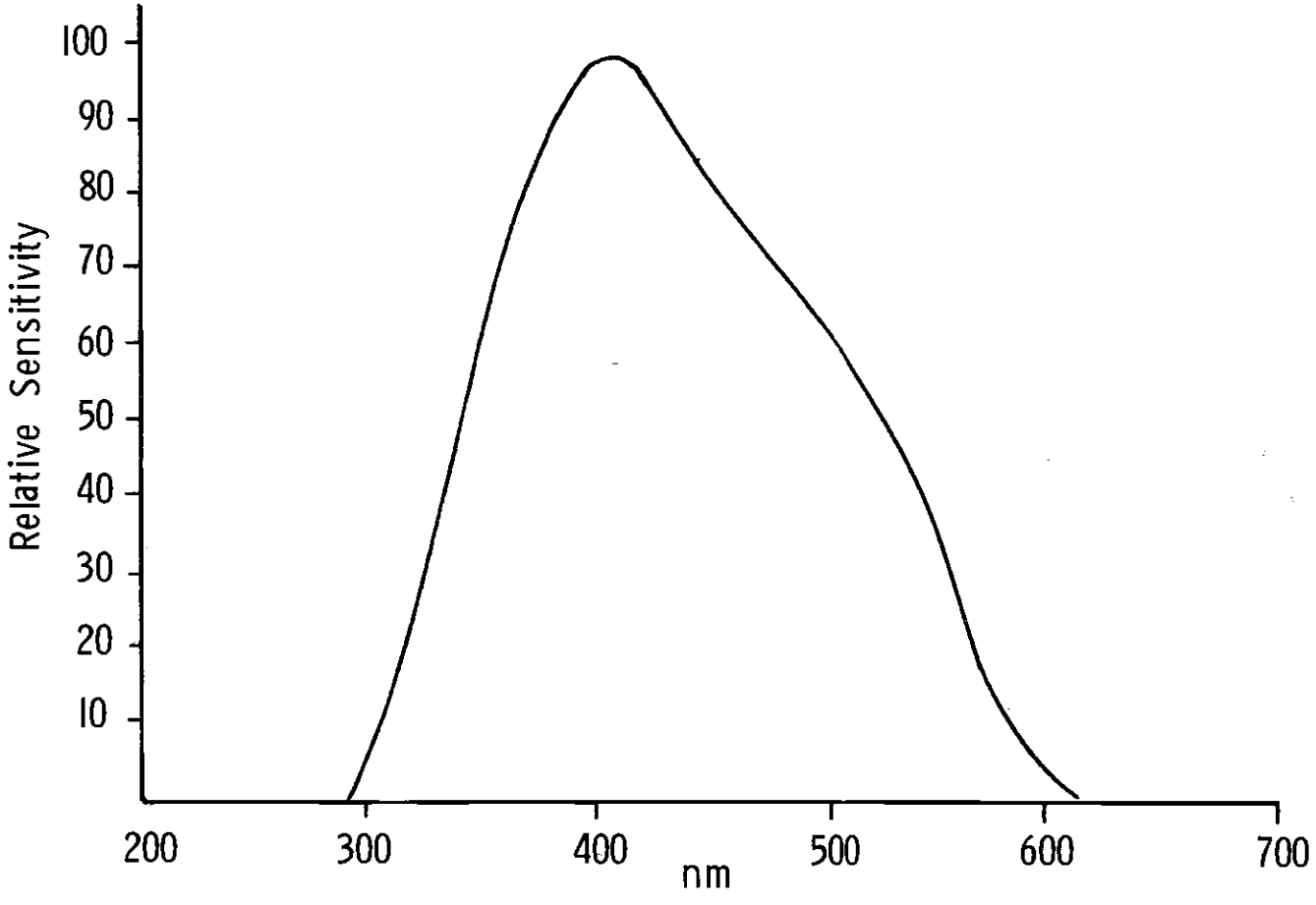


Figure 4. Response Curve of RCA-931A Photomultiplier Tube

which will fit inside the coupler, thereby permitting the future use of the remaining modules in a hybrid photometer.

Section II: Optoelectronic Photometer

The analyst can use a simple and inexpensive photometer in the determination of one particular species on a routine basis, or for use in the field. Here recent developments in the application of optoelectronic devices have made possible the realization of some very simple instruments (19), and a rather more complex, but equally dependable and inexpensive instrument, described here.

The photometer is shown in Figures 5a and 5b. A very complete discussion of the instrument, its design and testing, and its operating characteristics can be found in the literature (19). Here only a very brief discussion will be given.

The optoelectronic photometer was designed with a rather simple thought in mind. The situation in long path photometry is this. Unless perfrectly collimated light is used losses of radiant energy are unavoidable. With certain instrumental settings, as the cell length is increased a point is reached at which the energy received by the detector is insufficient to set 100% transmittance with the blank in the cell. After much discussion, the approach of driving the LED with a low duty cycle ampere level pulse was settled on. A gain of an order of magnitude in intensity can be realized in this way.

Collaterally, tuned detector circuits were incorporated into the planned design. Such circuits would serve to exclude the influence of ambient light of all types, a great benefit to the experimentor.

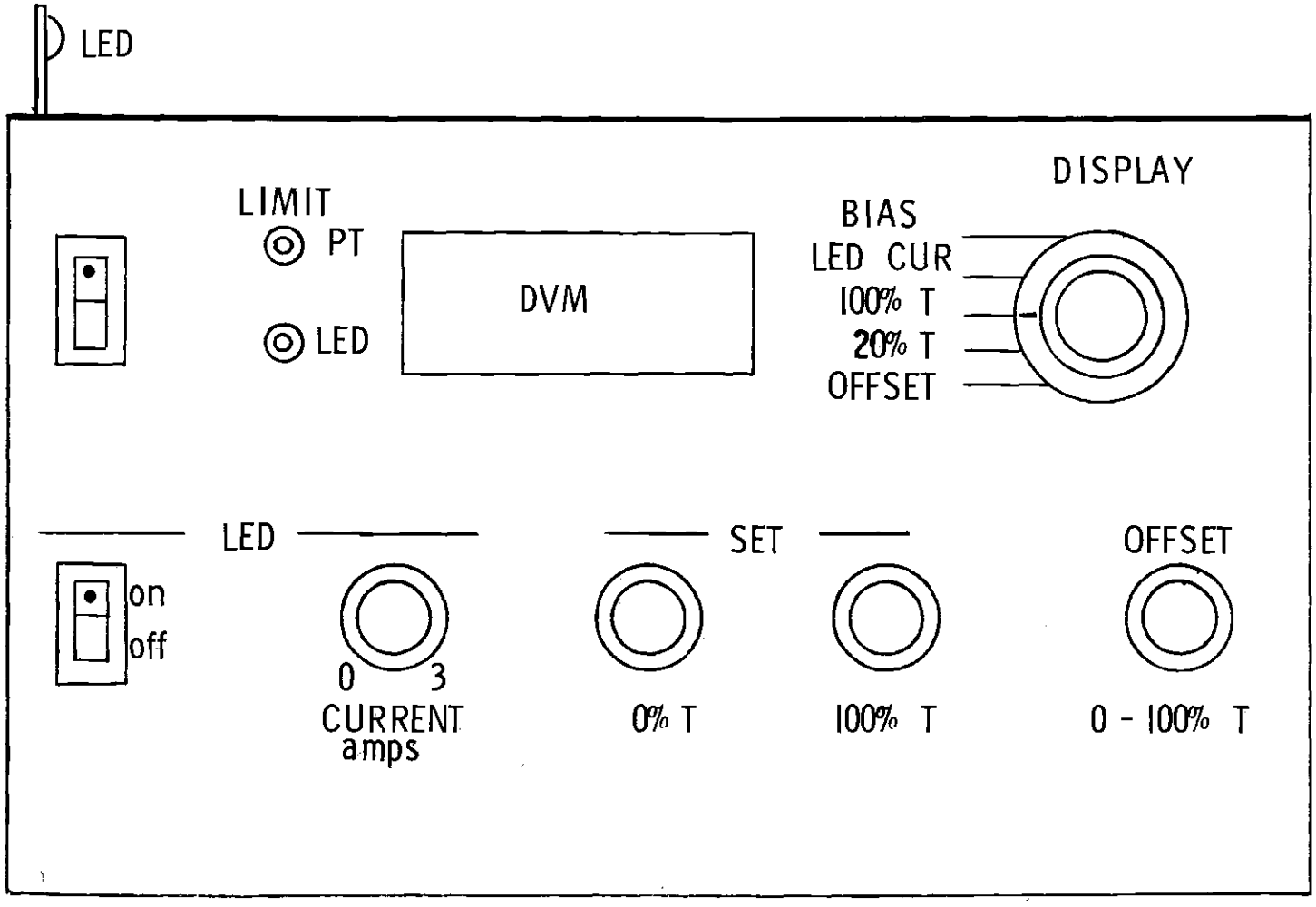


Figure 5a. Optoelectronic Photometer, LED and Circuitry Compartment

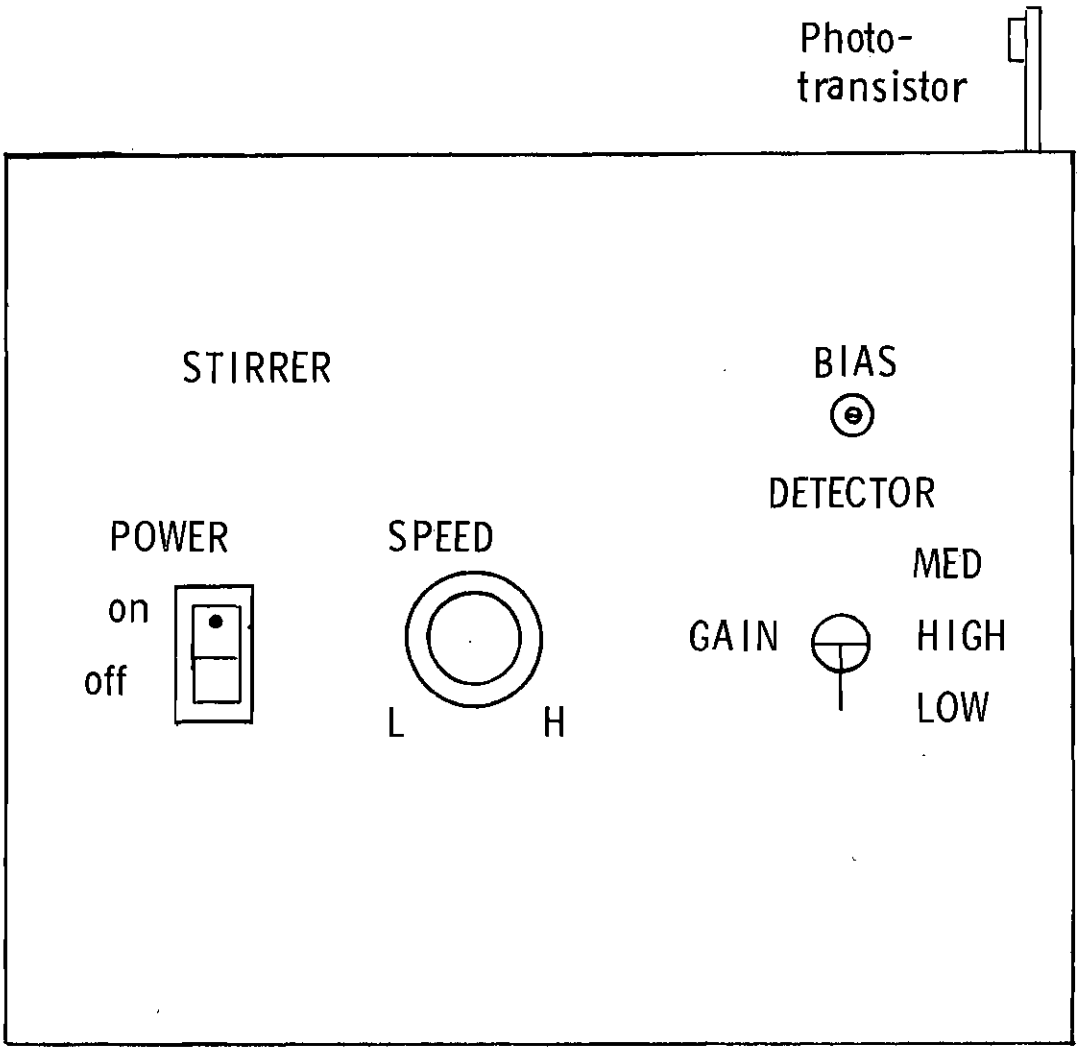


Figure 5b. Optoelectronic Photometer, Detector and Stirrer Compartment

The light emitting diode used as a light source is a Hewlett-Packard 5082-4658, with a peak emission at 635 nm. The phototransistor used as a detector is a Fairchild FPT-120. All other electronic components are of state-of-the-art quality.

Section III: Long Path Microcell

The problems associated with the design, construction, and application of long path microcells have been discussed by Flaschka and Coulter (10). The cell used in the work described in Chapter IV was constructed by Coulter and the details of its construction can be found in the literature (16). The microcell has an internal volume, that is, the volume from window to window, of 0.175 ml but because of the filling and emptying tubes the minimum volume required for filling is about 0.3 ml.

When using this cell a rather small volume of solution is exposed to a large surface of glass in the microcell and the possibilities of the sought for substance being either adsorbed onto or impurities being leached from the surface deserve attention. The following simple calculations describe the situation and yield the rather interesting result that the critical parameter, the surface area-to-volume ratio, is, in a first approximation, independent of cell length, depending only on the internal radius. If one considers a cylindrical cell with internal radius "r" and length "b", and neglects the surface area and volume pertaining to the filling arms, then the surface area in contact with the sample preparation can be represented as

$$S = 2\pi r^2 + 2\pi rb$$

where the first term represents the surface area of the cell windows and the second term that of the body. A simple rearrangement yields

$$S = 2\pi r(r+b)$$

The volume required to fill the assumed cell can be given as

$$V = \pi r^2 b$$

The surface area-to-volume ratio, R, that is, the number of square centimeters of solution surface exposed to the cell wall material per milliliter solution, is

$$R = S/V = 2\pi r(r+b)/\pi r^2 b$$

$$R = 2(r+b)/rb$$

For long path cells of great length with rather very small radius this simplifies, under the assumption that $r<<<b$, to

$$R = 2/r \text{ cm}^2\text{ml}^{-1}$$

Thus, the value for R for a cell with a 1 mm radius is

$$R = 2/0.1 = 20 \text{ cm}^2\text{ml}^{-1}$$

regardless of the length of the cell. For comparison, the R-value for a common 1-cm cell filled to a height of 2 cm is 4.5. Therefore, roughly five times as much exposed surface exists in the microcell.

CHAPTER IV

DETERMINATION OF MANGANESE

Introduction

Manganese was first recognized as an element in the 1770's, but for centuries before that time its compounds had been known and used. Manganese is important as an alloying element in steel, a basic part in the manufacture of dry cells, and as an additive in glass making. It was one of the first metals recognized as an essential trace element in both animal and plant metabolism. Thus, it is understandable that many methods have been developed for the detection and determination of manganese and its compounds and that analyzing for manganese is a continuing necessity.

The earliest methods for the determination of manganese were developed by chemists working in the steel industry. Most were titrimetric techniques, involving the oxidation of manganese to permanganate and its subsequent titration with a suitable reductant. The titration was visual, usually self-indicating, with the end point signalled by the disappearance of the purple permanganate color. These early titrimetric procedures are able to determine fairly low concentrations of manganese, are accurate and precise, and not interferred with by too many metals. Unfortunately, chromium, another important alloying element, is an exception. The method developed by Volhard (21) is considerably more

interference free, but is lengthy and involved. With the exhaustion of high grade manganese ores, the various industrial users were forced to use lower grade ores with a much lower manganese content. Also, special steels with much lower manganese contents were being manufactured. Therefore, it became necessary to find methods capable of determining smaller amounts of manganese at lower concentrations. The developments in clinical and environmental studies required further expansion of the limits of detection and dilution. To meet these challenges, analysts turned to colorimetry and then photometry.

Since the permanganate ion has a very intense purple color, it was only natural to base the earliest colorimetric techniques upon the comparison of the color intensities of unknown and standard permanganate solutions. As instruments were developed that could measure the absorbance of a solution at a chosen wavelength, photometric techniques were developed. Permanganate has a molar absorptivity of 2.3×10^3 liters per mole-cm at 545 nm, which is high for an inorganic entity. The classic photometric determination of manganese involves the silver ion catalyzed oxidation of the lower oxidation states of manganese to permanganate by persulfate. The persulfate method can not tolerate the presence of even very small amounts of chloride ion, so unless the solution is chloride free, it is necessary to remove completely any chloride that may be present. The complete destruction of any excess persulfate is also necessary, and there are difficulties associated with the color stability. Willard and Greathouse (22) greatly improved the situation with the introduction of periodate as the oxidant. No silver catalyst

is necessary and thus the presence of some chloride can be tolerated. Excess oxidant need not be destroyed and the color therefore becomes stable. It is thus possible to store oxidized standard solutions. In the case of steel and related analyses, the addition of sizeable amounts of phosphoric and sulfuric acids is necessary to prevent the precipitation of the sparingly soluble iron(III) periodate.

There are numerous other oxidants available for use in a variety of permanganate techniques, but the methods all suffer also from two serious common weaknesses, namely the relatively low molar absorptivity of the permanganate ion and the chloride ion interference. This is not to imply that these techniques are not useful. They have been refined and modified for use in certain situations, such as in the steel, glass making, and other industries. Chemists have learned to accept the limitations of these techniques, use them routinely, and even as standard methods for the determination of manganese. However, the low absorptivity and the chloride interference make application in certain other areas, e.g., environmental or clinical analysis, difficult if not impossible. The requirement of boiling the solution to develop the permanganate color is not conducive to field application. It is, therefore, understandable that many new methods were developed and tested. Some of these methods involved inorganic entities, such as the manganese(III) sulfato complex, while other utilized organic chromogenic agents, such as formaldoxime or benzidine. Most of these attempts achieved some success, especially with regard to higher absorptivity,

but none were any easier to perform or freer from interferences than the permanganate methods.

Bruno (23) developed a very simple and straightforward method for the determination of manganese via the manganese(III) triethanolamine complex. Although the method was developed over twenty-five years ago, it has not received the attention it would seem to merit.

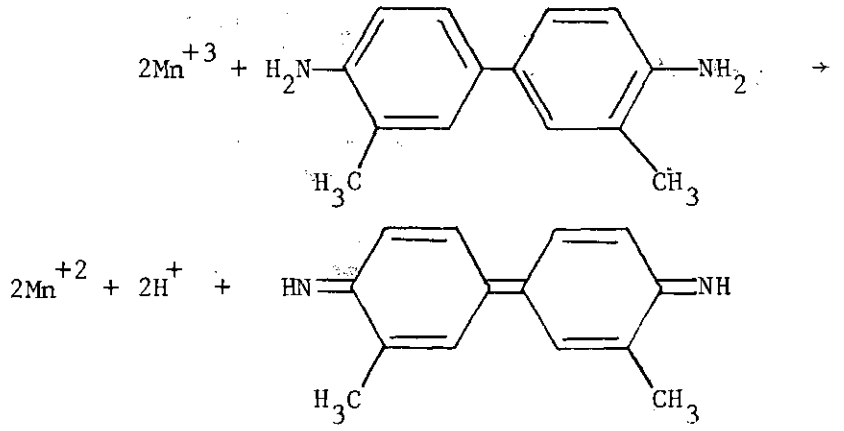
An emerald green complex is formed when manganese(II) is oxidized to manganese(III) by air in alkaline medium in the presence of triethanolamine (TEA). The oxidation is achieved by merely shaking or stirring the solution. The absorbance of the TEA complex is then measured. The method is interfered with by very few metals, chloride can be tolerated in any amount, and no boiling is required. It would seem to be the ideal situation, but unfortunately the molar absorptivity of the manganese(III)-TEA complex is only 2.5×10^2 liters per mole-cm at 615 nm, which is about one-tenth that of the permanganate. The simple Bruno method, however, offers great promise for a combination with long path photometry. In this way, by simply using a 10 or 15-cm cell, absorbance readings equal to those of the respective permanganate methods in a 1-cm cell would result, but without the shortcomings inherent to those methods.

Thus, the TEA procedure allows the equalization of the sensitivity and detection limit with those of the permanganate in a 1-cm cell but with great simplification and less interference. But, of course, even with the longest possible cells, it can never be brought to a level where it can satisfy the demands of micro and trace analysis, that is,

the determination of manganese in the μg/ml and lower levels. However, a paper by Barek and Berka (24) seemed to offer a clue for the modification and expansion of the Bruno method to allow determinations at these low levels.

Barek and Berka did not consider anything even remotely related to the determination of manganese, but rather were interested in the standardization of ascorbic acid solutions using potassium dichromate as the primary standard. The reaction between the dichromate and ascorbic acid does not proceed without unwanted side reactions, so Barek and Berka decided to try an indirect path. They allowed the dichromate to oxidize manganese(II) in the presence of pyrophosphate to manganese(III), which proceeds in a regular and reproducible manner. The manganese(III), stabilized as the pyrophosphato complex, can now react reproducibly, and free from side reactions with ascorbic acid in a titration. As indicator, some o-tolidine was added, which is oxidized by manganese(III) to an intensely yellow colored compound. At the end of the titration it is reduced to the colorless original o-tolidine. The aforementioned clue for an improvement in the manganese determination is the reaction between the manganese(III) and the o-tolidine. All that is needed is to allow the manganese to undergo the oxidation to manganese(III) in the presence of TEA and then to react it with o-tolidine and an intense yellow color would result.

The reaction between manganese and o-tolidine is



(IV-1)

The quinonediimine formed is intensely yellow, with a molar absorptivity of about 3.4×10^4 liters per mole-cm at 440 nm. The absorbance versus wavelength curve is presented in Figure 6. As can be seen in reaction IV-1, the oxidation of o-tolidine requires two electrons, whereas the reduction of manganese(III) to manganese(II) provides only one electron. Therefore, two manganese(III) ions are required for each molecule of o-tolidine. Thus, in relation to manganese, the effective molar absorptivity is only about 1.7×10^4. However, since even the lower effective molar absorptivity is about seven times higher than that of the permanganate, great improvement in the sensitivity is the result. If a long path instrumental finish were applied, a sensitivity of a few ng/ml should be obtainable. A few simple calculations show that using only a 1-cm cuvette, a desired absorbance reading of 0.5 corresponds to a manganese concentration of 3×10^{-5} M or about 1.5 µg/ml. If one accepts lower absorbance readings or employs a long path cell, it is easy to envision an even higher sensitivity. Therefore, it was decided to attempt the development of a method based on the manganese(III)

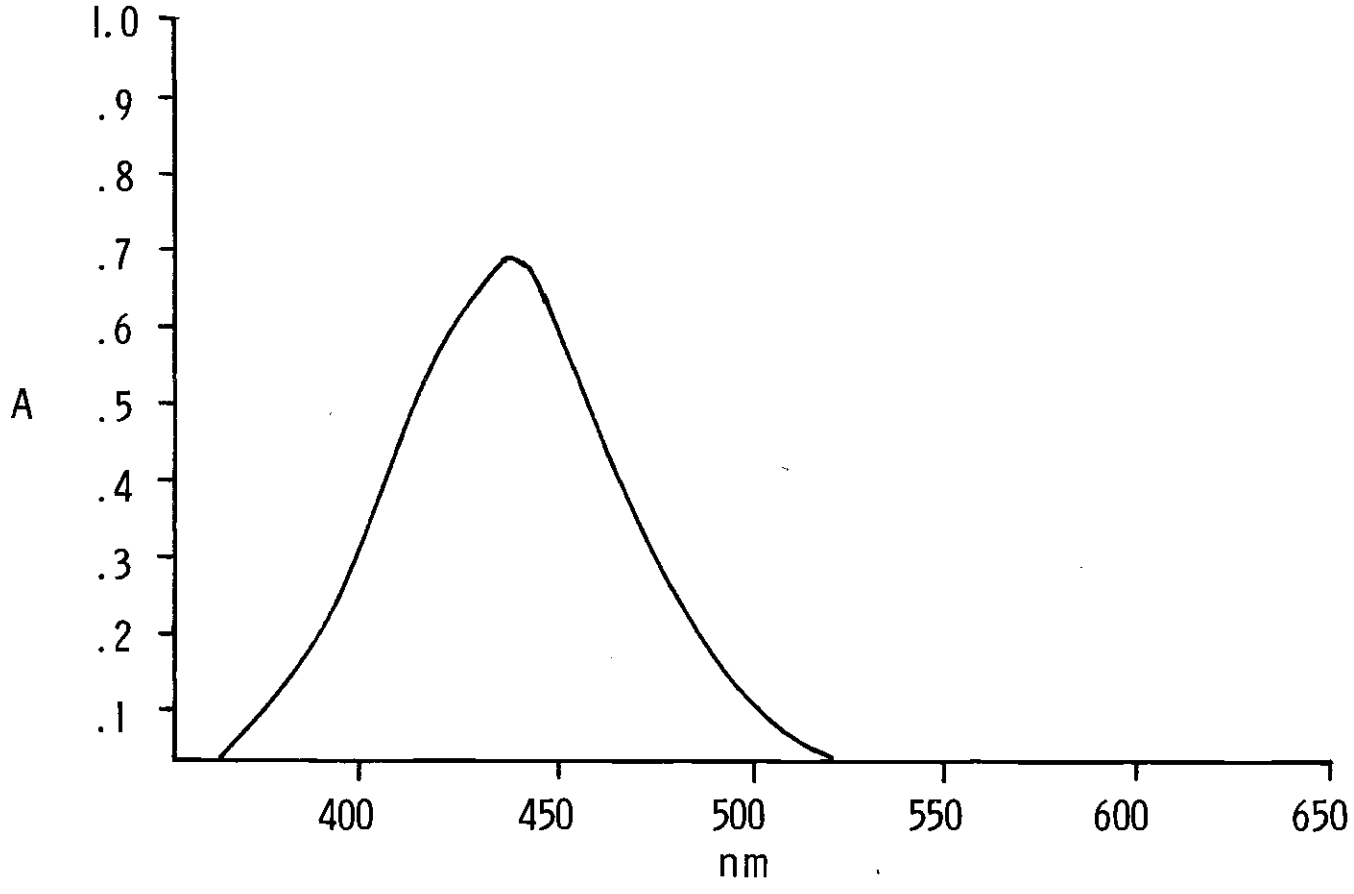


Figure 6. Absorbance Curve of the Quinonediimine of o-Tolidine

reaction with o-tolidine, with the manganese(III) formed by oxidation of manganese(II) in alkaline medium in the presence of TEA, significantly different from earlier approaches (25). The final goal was a method sensitive enough for use in environmental and clinical analysis, simple enough for use in the field, and rugged enough to ensure applicability by personnel with limited training.

The essential factors that must be considered in the development of any analytical method have been discussed in length in Chapter I. Here, where the thought is to develop a more sensitive method, there are additional considerations to be dealt with. The number of manipulations must be held to a minimum, reagent and other solutions should be combined where ever possible, and so on. The development procedure, with comments where necessary, will be discussed in the following section.

Section I: Development of the Method

The portion of the method pertaining to the oxidation according to Bruno was already known to be simple and straightforward, but was untested at the low levels of manganese to be encountered here. However, investigations by Flaschka, McClure, and Hornstein (26) using a cell with a very long path showed that the Bruno method works well even at these levels. The next steps involved the search for the conditions necessary for the reaction between o-tolidine and manganese(III) to take place.

The obvious approach, namely the mere addition of o-tolidine solution to the manganese(III)-TEA solution was not felt to be viable

because the oxidation of o-tolidine requires an acidic medium. A few simple experiments readily showed that no oxidation of the organic reagent takes place in alkaline medium. Therefore, the following procedure was tested. To the alkaline solution containing the manganese(III)-TEA complex was added o-tolidine and then sulfuric acid. A very short-lived blue color was observed, followed by the appearance of a faint yellow color. The blue color stems from the formation of the meriquinoid of o-tolidine. An investigation of the possibility of using the meriquinoid color for the determination of manganese is summarized in Section VI. It was obvious that the intended reaction, namely the formation of the quinonediimine, was not proceding as desired, so a change in the order of addition was made. First the acid was added to the manganese(III)-TEA solution and then the o-tolidine. A very intense yellow color developed immediately, but unfortunately it was neither stable nor reproducible. Obviously, the acidification leads to a disruption of the manganese(III)-TEA complex and the liberated manganese(III) does not react in a regular sequence with the o-tolidine. It is known that free manganese(III) will undergo a rapid disproportionation in acidic medium

$$2Mn(OH)_3 + 2H^+ \rightarrow Mn^{+2} + MnO_2 + 4H_2O \qquad \text{(IV-2)}$$

This reaction need not necessarily be responsible per se for the inconsistencies encountered. After all, the manganese(IV) formed could react with the o-tolidine without any electrons being lost. The

respective half-reaction is

$$MnO_2 + 4H^+ + 2e \rightarrow Mn^{+2} + 2H_2O \qquad E = 1.23v \qquad \text{(IV-3)}$$

It is probable that the high potential and the fact that a two electron step is now involved lead to side reactions with the result that the yellow oxidation product is not formed reproducibly and in strictly equivalent amounts. It seemed, therefore, necessary to stabilize the manganese(III) liberated from the TEA complex upon acidification in order to avoid the disproportionation. One can, as in the earlier works by Barek and Berka, and Flaschka and Yarbro (27,28), stabilize the manganese(III) in acid solution as the pyrophosphato complex, but another possible way of such stabilization is by the addition of a large amount of sulfuric acid. In the latter approach, the number of reagents would be kept to a minimum.

It is known that large amounts of manganese(III) can be stabilized as the sulfato complex in 4 to 7 F sulfuric acid (29). The manganese(III) forms a reddish colored complex with the HSO_4^- anion, and this complex forms the basis for the photometric determination of manganese at somewhat higher levels (30). Unfortunately, the TEA complex exists at pH 10, so the transition to a solution 7 F in sulfuric acid is a problem. In order to keep the solution volume at a manageable level, it is necessary to add rather highly concentrated sulfuric acid. Then, so much heat is evolved that the procedure had to be abandoned as impractical. It was therefore decided to resort to the original approach of stabilization employed by Barek and Berka, namely via pyrophosphate.

When pyrophosphate was added to the manganese(III)-TEA solution and the solution acidified, a very faint pink color was observed, indicating the presence of the manganese(III) ion. Upon addition of o-tolidine, the expected intense yellow color was produced. Next the quantitative aspects were studied.

A series of manganese(II) solutions were prepared with the concentration known to within 10% and taken through the tentative procedure. TEA and 9 F NaOH were added and the oxidation effected. Then pyrophosphate was added, the solution acidified, o-tolidine added, and the resulting solution was made to mark in a volumetric flask. The absorbance of the yellow color was measured at 440 nm using a Spectronic 20 and a standard cuvette. The resulting plot was found to be linear over the range of approximately 0.2 to 2 µg/ml manganese. These first studies were carried out with strict attention to the time elapsed between addition of the o-tolidine and the absorbance measurement. In this way, time dependence could not influence the data. This was verified as no scattering of the points along the calibration line occurred. The time dependence was then studied by noting the changes in absorbance of a 1.6 µg/ml manganese solution over a period of two hours. The fading of the color is pronounced as can be seen from the absorbance curve shown in Figure 7. Within the two hour period the absorbance decreased by nearly 40%. No color instability had been reported by either Barek and Berka, or Flaschka and Yarbro. It was, however, quite evident that some reaction or reactions were occurring that caused the color to fade, and thought was given to these earlier works.

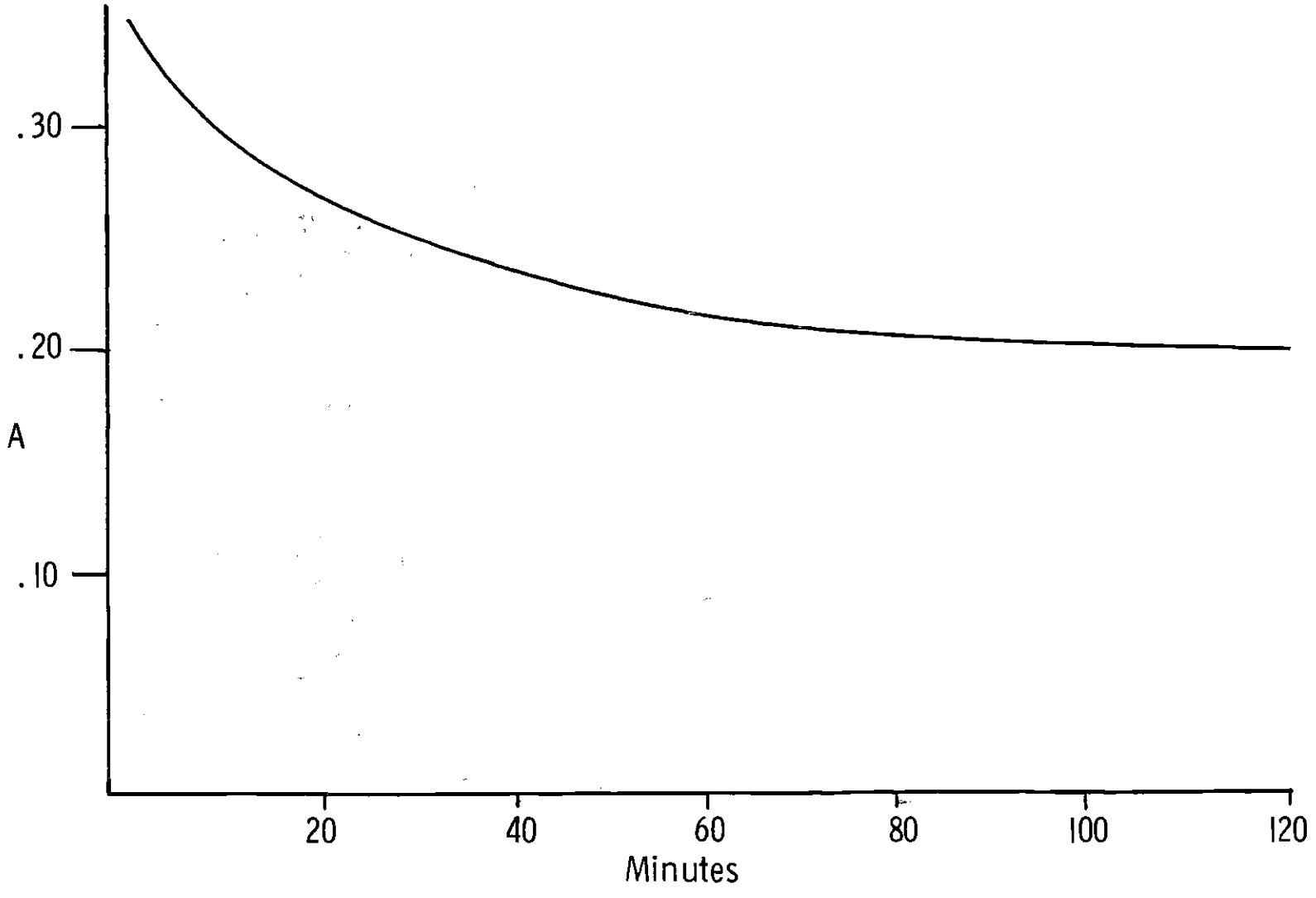


Figure 7. Time Dependence of Quinonediimine Color

Certain differences between the previously mentioned works and the proposed method came to mind. Neither of these had focused on manganese. Both methods, however, had used dichromate to oxidize manganese(II) to manganese(III) while reducing the chromium(VI) to chromium(III), prior to the reaction of the manganese(III) with the o-tolidine. It was decided, therefore, to introduce chromium(III) into the system under the hypothesis that its presence might somehow render the color stable. Various quantities of a chromium(III) solution were added, but no stabilization of the color was noted.

Since it was felt that the problem lay in either the transfer of the manganese(III) from the TEA complex to the pyrophosphato or in the reaction of the manganese(III) with the o-tolidine, subsequent studies were directed toward the investigation of these possibilities, with the former thought to be the more probable.

At first, 10 ml of a 0.01 F EDTA solution were added to the solution to retain the stability of the manganese(III) and prevent any undesired side reactions from occurring. In order to ensure the formation of the manganese(III)-EDTA complex, it was necessary to raise the pH of the solution but such a step proved unfeasible. The blue meriquinoid was formed initially, and then converted slowly and irreproducibly to the yellow quinonediimine. The additions of other volumes of the EDTA solution were tried, but as can be seen from the curves in Figure 8, this did not change the situation.

The only other difference between the works of Barek and Berka, Flaschka and Yarbro, and the method being developed was in the amount of

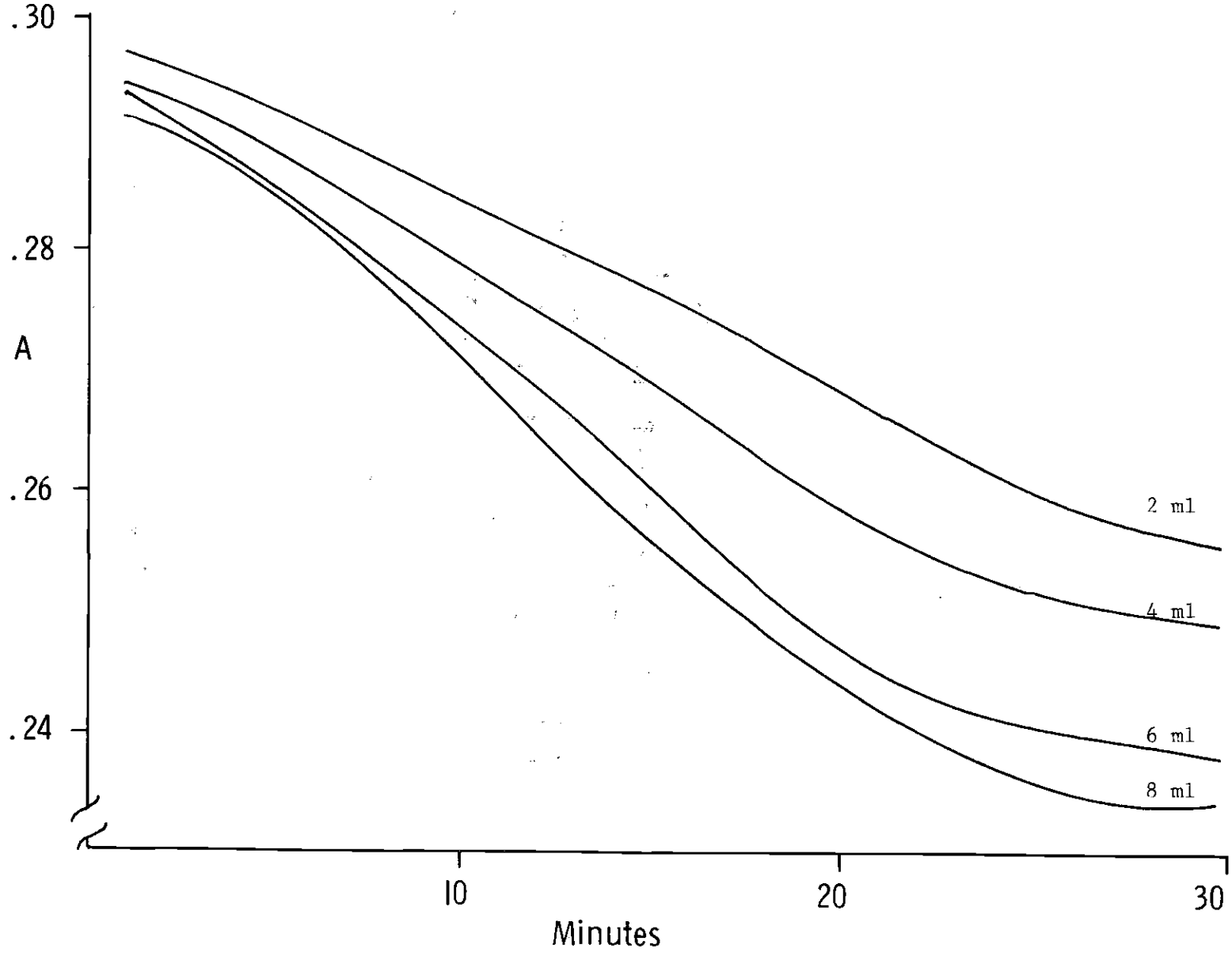


Figure 8. Effect of EDTA Upon Quinonediimine Color Stability

pyrophosphate added. These earlier works used a rather large amount of pyrophosphate, which was decreased in the procedure under development here. It was felt that the earlier papers used a large amount of pyrophosphate because of the attendant large amount of manganese. In the present situation, however, the amount of manganese was much smaller; consequently it was felt that the amount of pyrophosphate added could be decreased while still being in excess over the manganese. In light of the difficulties being encountered it was decided to increase the amount of pyrophosphate added, in hopes that a gross excess would prevent any manganese(III) from undergoing any unwanted side reactions during its transfer from the TEA complex to that of the pyrophosphato. A saturated solution of pyrophosphate was prepared by merely adding sodium pyrophosphate decahydrate to water until undissolved solid remained. Some of this solution was added to the solution containing the manganese(III)-TEA complex, the resulting solution was acidified and allowed to stand for 5 minutes. Then o-tolidine solution was added and the absorbance measurement made. This rather crude initial study showed that the approach greatly reduced the time dependence of the color intensity. Several studies were carried out on a solution 1.6 μg/ml in manganese to determine the optimum volume of the saturated pyrophosphate solution required. The resulting curves are shown in Figure 9. The tests started with the addition of 2 ml of the saturated pyrophosphate solution, and some stabilization of the color was noted. The additions were increased in 2 ml increments, with greater color stability noted as the volume of pyrophosphate solution added increased. However, with the

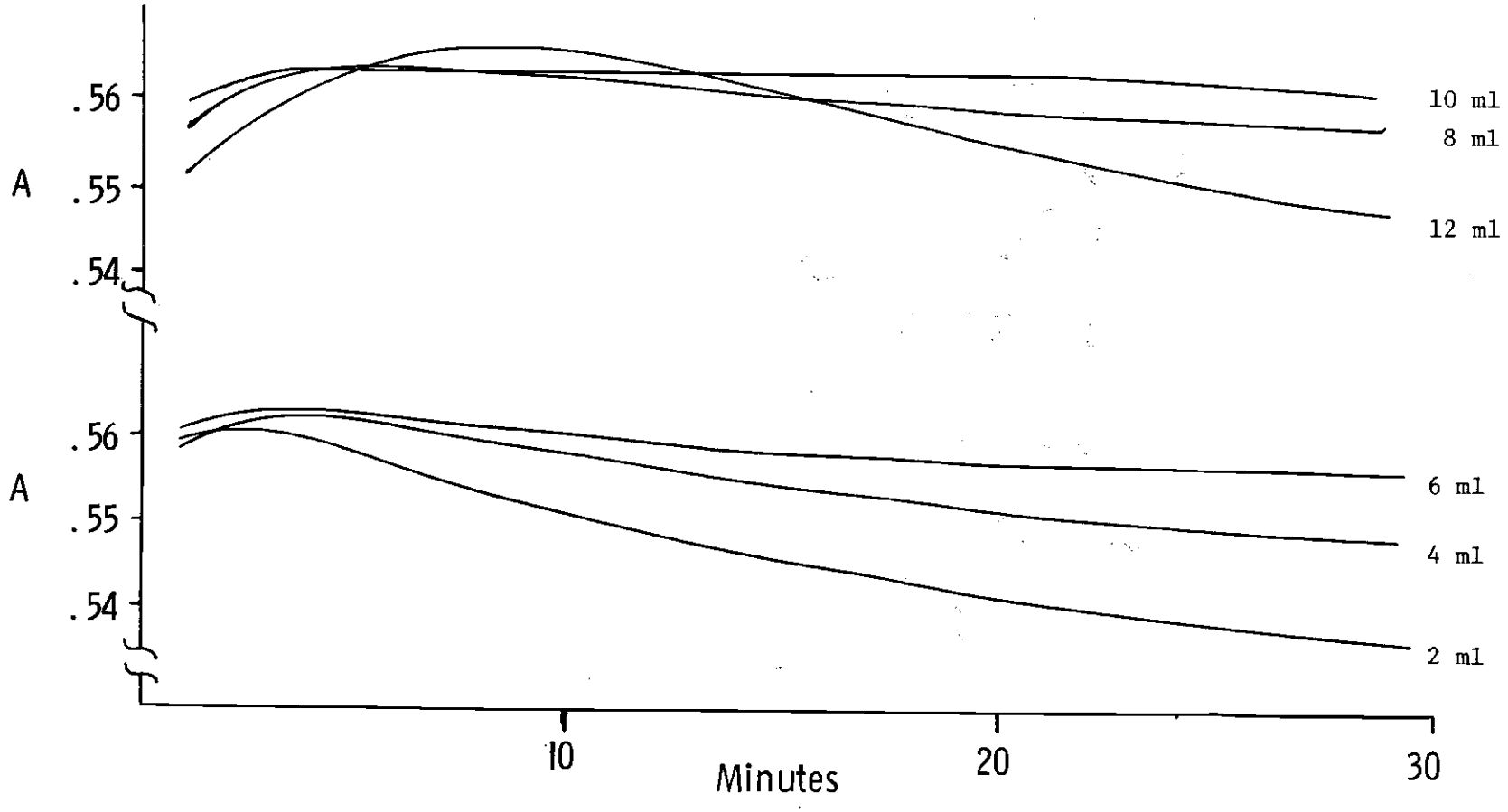


Figure 9. Effect of Pyrophosphate Upon Quinonediimine Color Stability

addition of 12 ml of the saturated pyrophosphate solution, a decrease in the color stability was noted. A further decrease was noted when 14 ml of the pyrophosphate solution was added. No additions above 14 ml were tried. The occurrence of the blue color due to the meriquinoid was noticed with the addition of both 12 ml and 14 ml of the saturated pyrophosphate solution. It existed for only a few seconds before the reaction proceeded to yield the quinonediimine. The increased pyrophosphate concentration raised the pH of the solution, so it became necessary to also study the volume of the 50% v/v sulfuric acid solution added, just as the pyrophosphate volume had been examined. For a final volume of 50 ml, the optimum volume of pyrophosphate was found to be 10 ml; above a minimum addition of 1 ml sulfuric acid no changes were observed. Subsequent studies were carried out to ensure the reproducibility of the absorbance values. At this time, accurate manganese standard solutions were prepared in order to better establish the concentration range over which Beer's Law was followed. These studies were carried out using a Spectronic 20 with its standard round cuvette. Later on the modular photometer and long path cell described in Chapter III were employed.

Section II: Water, Chemicals, and Equipment

Water

Further treatment of the water beyond distillation and deionization is necessary before it can be used in the preparation of any solution if tap water is used as the original source. Such water is treated with either chlorine or chloramine-T. As a consequence of this treatment, chlorine stemming either from that already present in the water or that formed from the chloramine-T during the distillation, will

be present in the distillate and will react with o-tolidine to produce the quinonediimine; in fact the reaction serves as the basis for the determination of chlorine in water. It is therefore necessary to ensure that these species are absent. It would seem that the presence of these species would be accounted for in the blank, but this is not the case, so their removal is necessary. The removal can be effected by boiling the previously distilled and deionized water for a few minutes.

Chemicals

All metal salt solutions were prepared from Baker Analyzed salts. Eastman-Kodak o-tolidine and 8-hydroxyquinoline were used in preparation of solutions of these substances. The TEA used was Fisher Spectranalyzed grade reagent. All acids and bases used were prepared from reagent grade stock. Any solvents used were also reagent grade.

Equipment

Class A volumetric glassware was used without further calibration. Other glassware, such as beakers and flasks, was used as necessary.

The initial development of the method used a Spectronic 20 spectrophotometer. The long path experiments used the modular photometer described in Chapter III.

Section III: Procedural Details

Reagents and Solutions

Pyrophosphate Solution, saturated. Add 12 g $Na_4P_2O_7 \cdot 10H_2O$ to 100 ml water. Make certain that undissolved solid remains. Although the hydrolysis of the pyrophosphate ion is slow in cold neutral or

alkaline solutions, it is advisable not to use solutions that are over 2 or 3 weeks old.

o-Tolidine Solution. Add about 0.1 g o-tolidine to 1 ml concentrated HCl and bring to a total volume of about 100 ml with water. This solution has a shelf life of about 1 week.

Sulfuric Acid Solution, 50%.

Sodium Hydroxide, 9 F. Dissolve 36 g reagent grade NaOH to a total volume of 250 ml water.

TEA. Concentrated reagent grade TEA directly from the container.

Phosphoric Acid. Concentrated reagent grade H_3PO_4 directly from the container.

Iron(II) Solution, 0.1 F. Add 10 g Mohr's salt, $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$, to 250 ml water. Keep tightly stoppered. It is advisable not to use solutions that are over 5 days old.

Manganese Standard, 100 µg/ml. Dissolve 0.36024 g $MnCl_2 \cdot 4H_2O$ in about 100 ml water, add a few drops concentrated HCl, and make to mark in a 1-L volumetric flask with water.

Manganese Working Standard I. Add 100 ml of the 100 µg/ml manganese standard to a 1-L volumetric flask and make to mark with water. This solution will serve as the working standard for finishing with a 1-cm cell. It has a shelf life of about 5 days.

Manganese Working Standard II. Add 10 ml of manganese working standard I to a 1-L volumetric flask and make to mark with water. This solution will serve as the working standard for finishing with a 20-cm cell. It has a shelf life of about 3 days.

Sample Treatment

If the sample is a solid material, such as a steel, dissolve it in an appropriate manner. If the sample is a liquid, such as sea water or industrial effluent, remove any particulate matter. If a complicated organic matrix is present, as in the case of some effluents and natural waters or with a biological sample, it is necessary to remove the matrix. This can be done by precipitation of the metals as the oxinates, centrifugation and removal of the supernatant liquid, and then dissolution of the precipitate with acid. In certain cases, i.e. blood serum, ashing may be necessary.

The volume of the sample solution should be adjusted so that the manganese concentration is in the proper range for the analysis. Any dilutions should be made with water. The volume adjustments should be such that an aliquot of no more than 10 ml contains no more than 4 µg manganese for a finish with a 1-cm cell, or no more than 1.2 µg for a finish with a 20-cm cell. After any necessary volume adjustments have been made, check the sample solution for the presence of any interferences, in the order given below.

Unless vanadium is definitely known to be present, check the sample solution for it. Any accepted qualitative test, such as those of Feigl (31), can be used. If only small amounts of vanadium are present, they can be dealt with in the analysis. If vanadium is present in amounts over twice that of manganese, it is necessary to remove the vanadium. Any accepted separation, such as a cupferron extraction (32), can be used.

Next, any strong oxidants, such as cerium(IV), dichromate, chlorine, or bromine, must be dealt with. If such species are definitely known to be present in only small amounts, they can be dealt with by the addition of small amounts of the iron(II) solution as described in the procedures. The iron(II) is oxidized to iron(III), thereby reducing any oxidants present. If strong oxidants are not definitely known to be present, test for them by taking 1 ml of the sample preparation, adding 1 ml concentrated phosphoric acid, and 1 ml o-tolidine. If a yellow color develops, then an oxidizing interference is present. A simple qualitative test, such as one of those set forth by Feigl (31), can be used to establish an approximate concentration. If only a small amount of the oxidant is present, then it can be dealt with through the use of the iron(II) addition. If a large amount of the oxidant or oxidants are present, then treatment with an excess of sulfurous acid is necessary. Add a few drops (to excess) of sulfurous acid and then remove the excess by boiling. If boiling is impractical, prolonged aeration can be used.

If any higher oxidation states of manganese are present, it is necessary to reduce them to manganese(II), which will be automatically achieved by the addition of iron(II).

The solution should be adjusted to about pH 2 before beginning the procedure. This is to make the volume of 9 F NaOH prescribed in the procedure adequate to reach the required alkalinity. The preneutralization is simple even when heavy metals are present. Simply add NaOH until hydrous oxides begin to precipitate. Then add one or two drops of

concentrated acid to redissolve them. The sample solution is now ready to be carried through the analysis.

Procedure

1. Place into a 25-ml volumetric flask a known volume of the sample preparation (up to 10 ml). Chose the volume so that the manganese concentration in the final 25 ml solution is between 0.10 and 1.6 μg/ml or 2.0 to 45.0 ng/ml, for finishing with a 1-cm or 20-cm cell, respectively. Add 1 ml TEA, 1 ml 9 F NaOH, 1 ml 0.1 F iron(II) solution, stopper the flask and shake for 30 seconds. Reopen the flask to admit more air, restopper, and shake for another 30 seconds.

2. Add 5 ml of the pyrophosphate solution and 5 ml of the 50% v/v sulfuric acid solution, mix, and allow it to stand for 5 minutes.

3. Add 1 ml of the o-tolidine solution and make to mark with water. Stopper, mix well, and allow to stand for 5 minutes.

4. Measure the absorbance of the solution at 440 nm against the appropriate blank preparation and read the result from the respective calibration curve.

5A. Blank when vanadium is absent: instead of the volume of sample preparation taken in step 1, use an equal volume of distilled water and carry through steps 2 to 4. Use this solution to set 100% T.

5B. Blank when vanadium is present: into a 25-ml volumetric flask place 1 ml TEA, 1 ml 50% v/v sulfuric acid, 1 ml 0.1 F iron(II), 1 ml concentrated phosphoric acid, and 5 ml pyrophosphate solution. Then add exactly the same volume of sample preparation as was used in step 1. Mix well. Add 1 ml o-tolidine solution, make to mark with

water, mix, and allow to stand for 5 minutes. Use this solution to set 100% T.

6. Calibration curve for 1-cm cell finish: carry exactly 0.5, 2, and 4 ml working standard I through the procedure and plot the absorbance versus the respective final concentrations of exactly 0.2, 0.8, and 1.6 µg/ml manganese.

7. Calibration curve for 20-cm cell finish: carry exactly 0.5, 1, 4, and 8 ml of working standard II through the procedure and plot absorbance versus the respective final concentrations of exactly 2, 4, 16, and 32 ng/ml manganese.

Section IV: Results and Discussion

The first studies involved the more exacting establishment of the concentration range over which the procedure gives a linear absorbance versus concentration curve that passes through the origin. Preliminary studies, using the manganese solutions with concentrations known to only within 10% of the nominal value, had indicated a range of about 0.2 to 2 µg/ml manganese. Using the standard solutions prepared later, a range of 0.10 to 1.6 µg/ml manganese was established. Below 0.10 µg/ml, no signal above the noise level was detectable; above 1.6 µg/ml, non-linearity of the calibration curve became pronounced. A representative calibration curve is shown in Figure 10. A linear regression analysis of the points gave a degree of fit of 0.9993, confirming the linearity of the plot and showing a very good adherence to Beer's Law over the experimentally established manganese concentration range. After the

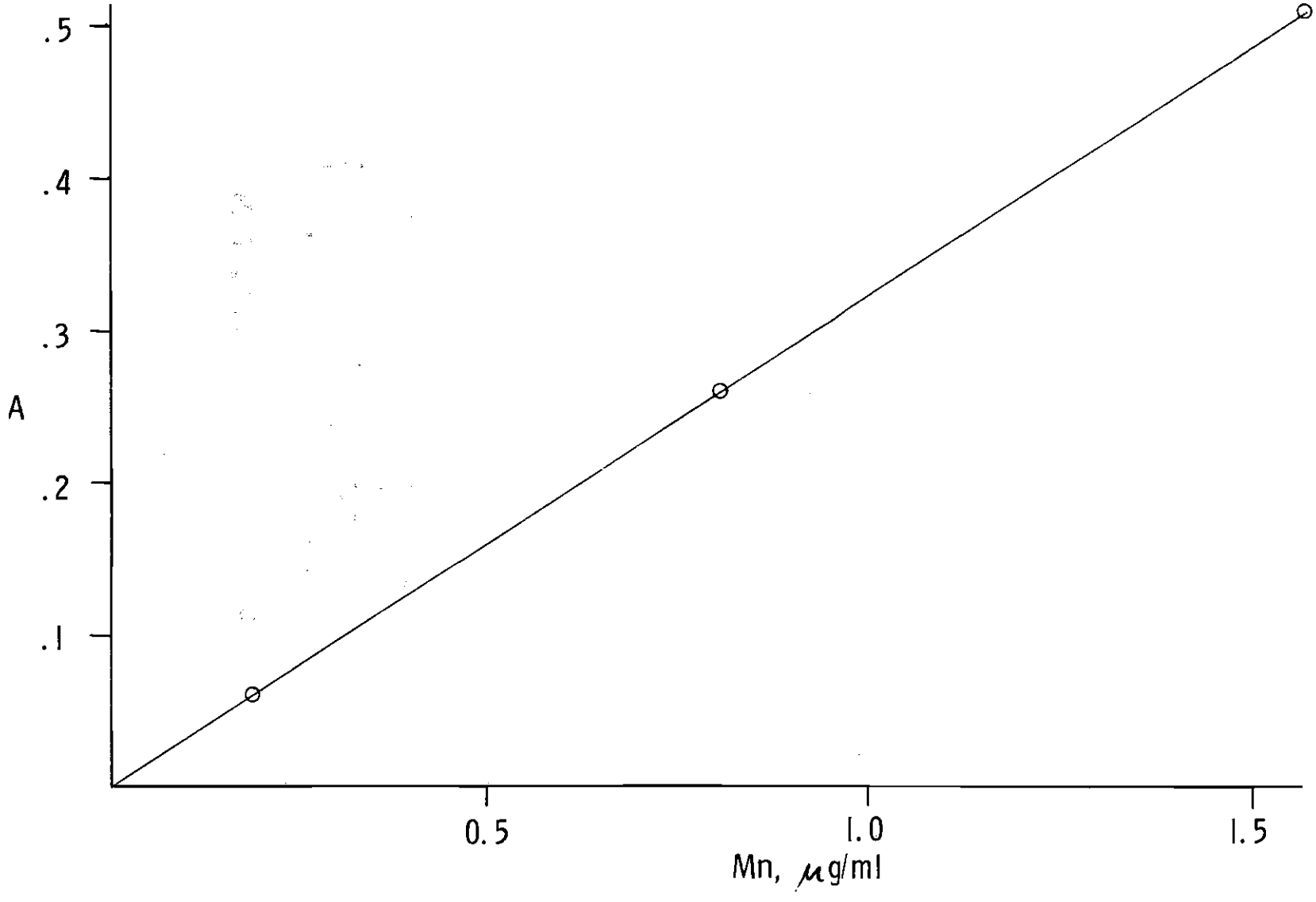


Figure 10. Photometric Determination of Manganese with o-Tolidine Using a 1-cm Cell

calibration curve was prepared, solutions from the same standards, but prepared by a third party and with manganese concentrations unknown to the experimenter, were carried through the analysis. These "unknowns" were used to further test the method. A summary of the results is presented in Table 1. The values have standard deviations comparable to those of the existing manganese methods involving levels that are at or even below the sensitivity limits of these methods. A test at the 99% confidence level shows no significant differences of the standard deviations within the replicate determinations.

Interferences

A study of possible interferences was also carried out at this stage, under the assumption that if a substance interfered at these levels it would, in all probability, even more interfere at the lower levels to be encountered in the long path finish.

Bruno found several cationic and anionic interferences. Nickel(II), cobalt(II), copper(II), and chromium(III) interfere because they also form colored complexes with TEA that absorb at the analytical wavelength. Magnesium(II) interferes because of the formation of an insoluble hydroxide, preventing a photometric finish. Bruno also found ammonium to interfere, but gave no reason. Investigation showed that the ammonium acted as a buffering agent, preventing the realization of the proper alkalinity. Citrate, oxalate, tartrate, cyanide, and thiocyanate were also said to interfere. Since all of these interferences are with regard to the TEA complex, a reexamination of the situation was necessary.

Table 1. Results of the Determination of Manganese with o-Tolidine Using a 1-cm Cell

Manganese, µg taken	*found	$\bar{X}$	S_x	Δ%
2.50	2.38, 2.42, 2.57, 2.50, 2.48, 2.53, 2.56, 2.45, 2.47, 2.52	2.49	0.06	-0.400
4.0	3.75, 3.87, 4.10, 4.05, 4.00, 4.05, 3.90, 3.95, 3.90, 4.10	3.97	0.114	-0.750
6.00	6.05, 5.85, 5.95, 6.02, 6.00, 6.02, 5.95, 5.95, 6.00, 6.05	5.98	0.061	-0.333
8.00	7.97, 7.90, 8.10, 8.02, 8.05, 8.00, 7.95, 8.00, 8.05, 8.05	8.01	0.086	0.125
10.00	9.92, 9.95, 10.10, 10.05, 10.05, 10.10, 10.15, 10.00, 9.90, 9.95	10.02	0.058	0.200
12.00	11.60, 11.85, 12.10, 11.95, 10.90, 12.00, 11.90, 12.95, 13.05, 11.90	12.02	0.617	0.167
16.00	16.05, 14.85, 16.70, 15.85, 15.95, 16.20, 16.05, 15.50, 15.75, 16.10	15.90	0.484	-0.625
25.00	23.50, 24.75, 25.80, 25.00, 23.10, 25.75, 26.25, 25.00, 25.50, 23.90	24.86	1.051	-0.560
30.00	30.00, 28.75, 27.50, 31.50, 32.70, 29.15, 29.70, 30.50, 33.25, 27.95	30.10	1.917	0.333
35.00	33.00, 35.50, 34.50, 36.20, 35.00, 35.50, 37.25, 35.00, 34.75, 35.00	35.17	1.110	0.486

*One more significant figure is given than can be justified. However, this was done because the data were subjected to a statistical analysis.

Any metals that form colored TEA complexes will probably exist as free ions in the acidic medium of the o-tolidine method. In any case, the TEA complexes are weakly absorbing when compared to the quinonediimine, and the wavelengths, 625 nm for the TEA versus 440 nm for the quinonediimine, are far enough apart to prevent any interference. An examination of the species listed by Bruno showed that they did not interfere here. It was, however, necessary to test several other species, namely any strong oxidants, and also iron(II) and iron(III).

Iron(III) may interfere through the formation of its chloro complexes, which are yellow colored. There may be chloride ion present in the original sample, it may be introduced during the dissolution of a metal, and it is introduced with the addition of the o-tolidine reagent. The yellow color of the chloro complexes of iron(III) is effectively masked by the presence of the pyrophosphate, so that iron(III) does not interfere.

Iron(II) was also examined and found not to interfere. This is because, in all probability, it is oxidized to iron(III) during the oxidation of manganese(II) to manganese(III). The fact that iron(II) does not interfere proved to be beneficial. By the addition of small amounts of an iron(II) solution during the procedure, any strong oxidant is reduced and rendered inactive.

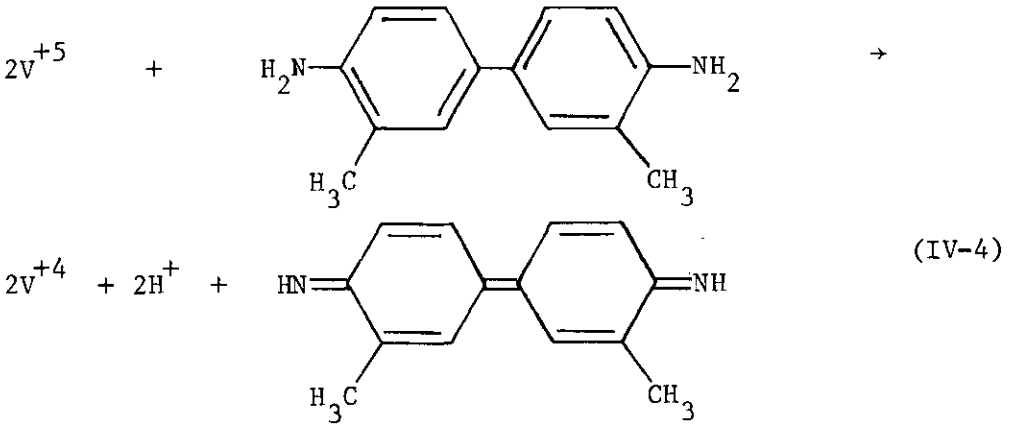
Chlorine and bromine are strong oxidants and do interfere, as do cerium(IV) and dichromate. If present in small amounts, such species can be handled by the addition of iron(II) as described earlier. Chlorine and bromine, if present in large amounts, can be removed by

merely boiling the sample solution. The others listed, if present in large amounts, must be reduced to a lower oxidation state, i.e., chromium(III) and cerium(III), which will not interfere. Sulfurous acid is the reductant of choice, since the unreacted excess can readily be expelled by boiling, or if unfeasible, by prolonged aeration.

Bruno found the organic species listed interfered at the 40 µg/ml level. Those species were tested here and found not to interfere even at 1000 µg/ml.

Vanadium. Vanadium(V) was known from earlier studies to be an interference (28). If it is present in large amounts, it is necessary to perform a separation. A cupferron extraction (32) is the recommended procedure. If only relatively small amounts are present, it can be dealt with in a rather unique way.

Research by Dobbs (33) has shown that vanadium(V) is capable of oxidizing o-tolidine



(IV-4)

It was suggested that a slight change in the procedure under study could afford a way to avoid the vanadium interference. Since manganese(II) is oxidized in alkaline medium to manganese(III), if the sample were added

to acidic medium, then the manganese should remain in the lower oxidation state and not react with the o-tolidine, leaving only the vanadium capable of reaction. The following procedure was tried. One solution, containing manganese(II) and vanadium(V), was carried through the analysis in the normal way. A second solution, containing only manganese(II), in an amount equal to that of the first solution, and no vanadium, was also carried through the analysis. A normal reagent blank was also prepared. A fourth solution was treated differently. TEA, sulfuric and phosphoric acids, and pyrophosphate were added. NaOH was omitted since it was not needed. To this acidic medium, the solution of manganese(II) and vanadium(V), in amounts equal to that of the first solution, was added. Then o-tolidine was added, the color allowed to develop, and the solution used as a blank for the first solution. The absorbance of solution II, when read against the reagent blank, should be the same as that of solution I read against solution IV, with solution IV acting as the blank. Several solutions, with varying vanadium(V) and manganese(II) concentrations were taken through this new procedure, and the results are given in Table 2.

No other interference studies were carried out. A complete summary of the interference study is presented in Table 3.

Long Path Finish

The next step was to find out whether or not the method could be successfully adapted to a long path finish, in order to further increase the sensitivity. The instrument and cell described in Chapter III were used for these measurements.

Table 2. Results of the Vanadium Blanking Procedure

Sample	manganese, µg/ml	vanadium, µg/ml	Absorbance
1	0.80	0.00	0.282
	0.80	2.00	0.280
2	1.60	0.00	0.526
	1.60	1.00	0.530
3	0.80	0.00	0.280
	0.80	0.40	0.282
4	1.60	0.00	0.530
	1.60	4.00	0.530

Table 3. Allowable Levels of Various Species for a Manganese Concentration of µg/ml

Species	Concentration, µg/ml
Fe(II)	1000
Fe(III)	1000
Ni(II)	1000
Cu(II)	1000
Co(II)	1000
Cr(III)	1000
Mg(II)	1000
NH_4^+	1000
CN^-	1000
SCN^-	1000
Oxalate	1000
Tartrate	1000
Citrate	1000
F^-, Cl^-, Br^-, I^-	1000
Br_2, Cl_2	<1
V(V)	<1
Cr(VI)	<1
Ce(IV)	<1

Theoretically, any increase in cell length would bring about an equal increase in the absorbance reading. With this thought in mind, it was calculated that the use of a 20-cm cell should make possible the determination of manganese at the 5 ng/ml level. Therefore the first long path studies were carried out in the 5 to 30 ng/ml manganese range.

These long path studies showed that the actual range had a lower limit of 2 ng/ml manganese and an upper limit of 45 ng/ml. An appropriate series of standards were prepared and the concentration range tested and verified. The calibration curve that resulted was linear over the experimentally established range and passed through the origin. A representative example is shown in Figure 11. This curve had a degree of fit of 0.9985, calculated in a linear regression analysis. This high degree of linearity confirmed adherence to Beer's Law.

Again, as had been done in the Spectronic 20 studies, a third party prepared a series of solutions with manganese concentrations unknown to the experimenter. These unknowns were carried through the analysis. The results are presented in Table 4. The standard deviations listed in that table are well within the limits of those of accepted methods for the determination of manganese at the µg/ml level. A test of the standard deviations at the 99% confidence level shows no statistically significant difference within the replicate determinations.

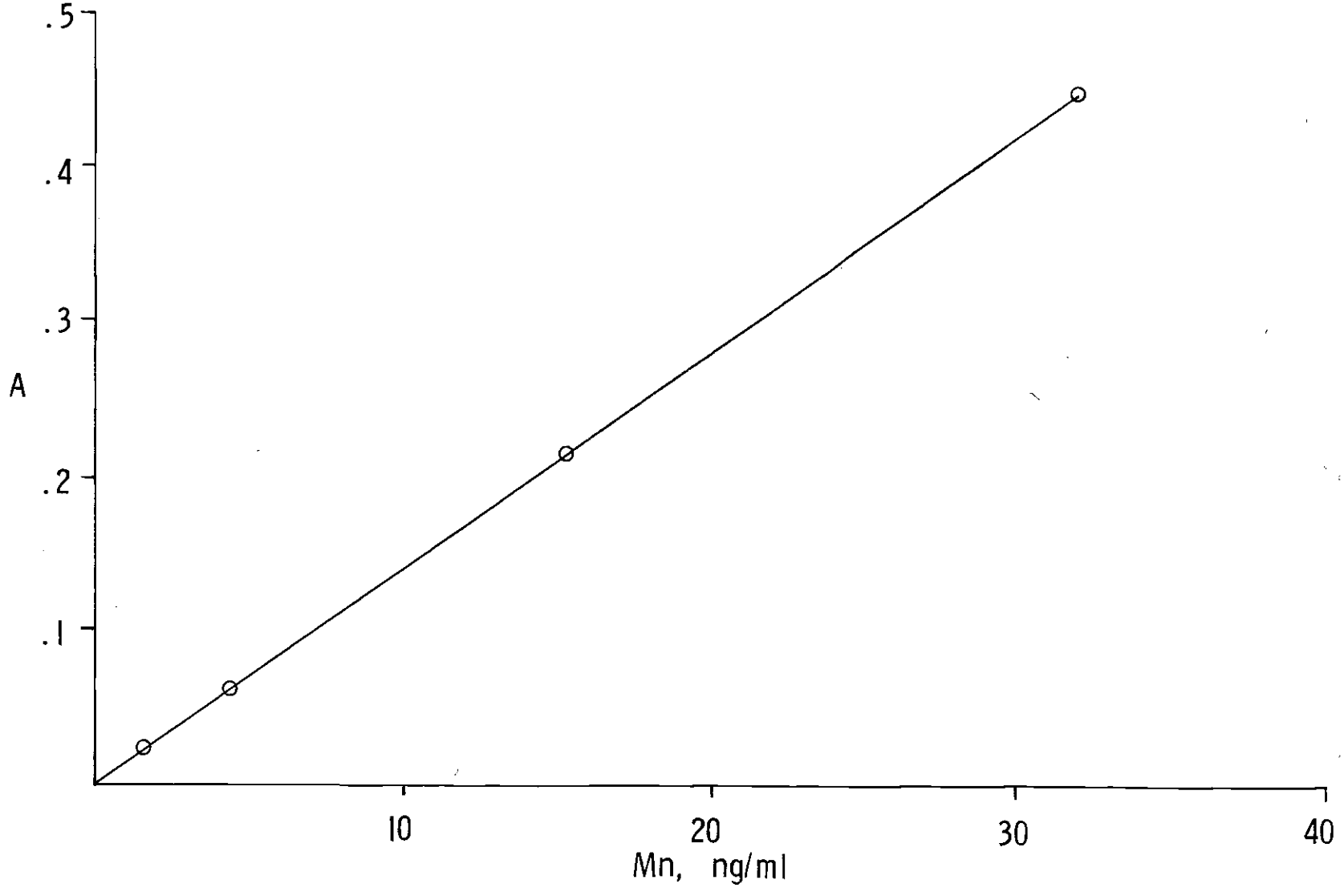


Figure 11. Photometric Determination of Manganese with o-Tolidine Using a 20-cm Cell

Table 4. Results of the Determination of Manganese with o-Tolidine Using a 20-cm Cell

Manganese, μg taken	*found	$\bar{X}$	S_x	Δ%
62.5	48.75, 53.75, 66.25, 70.00, 70.00, 67.50, 72.50, 62.50, 66.25, 67.50	64.50	7.574	3.201
125.0	122.5, 125.0, 132.5, 118.8, 122.5, 131.2, 125.0, 120.0, 128.8, 137.5	126.4	5.984	1.122
200.0	202.5, 215.0, 181.2, 205.0, 200.0, 198.8, 200.0, 202.5, 193.8, 200.0	199.9	8.535	-0.051
250.0	246.2, 247.5, 253.8, 268.8, 256.2, 237.5, 250.0, 251.2, 225.0, 255.0	249.1	11.676	-0.360
300.0	275.0, 293.8, 303.8, 300.0, 303.8, 295.0, 297.5, 305.0, 287.5, 300.0	296.1	9.164	-1.300
375.0	406.2, 380.0, 368.8, 372.5, 375.0, 362.5, 380.0, 382.5, 370.0, 377.5	377.5	11.782	0.667
500.0	493.8, 497.5, 500.0, 495.0, 537.5, 502.5, 468.8, 487.5, 512.5, 525.0	502.0	19.301	0.400
625.0	580.0, 637.5, 625.0, 656.2, 605.0, 637.5, 618.8, 675.0, 605.0, 625.0	626.5	27.135	0.240
750.0	750.0, 737.5, 743.8, 780.0, 762.5, 755.0, 831.2, 752.5, 750.0, 755.0	761.8	26.916	1.573
1000.0	925.0, 956.2, 980.0, 968.8, 937.5, 956.2, 1005.0, 1050.0, 998.7, 1050.0	982.8	43.191	-1.725

*One more significant figure is given than can be justified. However, this was done because the data were subjected to a statistical analysis.

Section V: Analysis of Seawater

Once the method had been successfully applied to laboratory solutions, it was decided to test its applicability to a "real" sample. The material chosen was sea water, collected from the Atlantic Ocean off the coast of Savannah, Georgia. Literature values (34,35) for manganese concentrations in Atlantic Ocean waters are reported to be from 2 ng/ml in southern waters to 10 ng/ml in northern waters. Unfortunately, there is also vanadium present in sea water, in the amounts of 2 ng/ml in southern waters to 4 ng/ml in northern waters. The technique given in the procedure for dealing with small amounts of vanadium would be applicable here, but it had not been developed when the analysis was conducted, and by the time the technique had been developed, the sea water sample had been exhausted. It was not worth the several hour trip to collect a new sample, and it was felt inadvisable to request that someone on site collect the sample and ship it to the investigator, since no control could be exercised over the taking of the sample. As a further consideration, Dobbs (33), working with other benzidine derivatives, had reported success in the development of a method for the determination of vanadium in the presence of manganese. As it is rare for a sample such as sea water to be analyzed for only one species, the procedure described here could be used to determine the sum of vanadium and manganese. The Dobbs method could be applied to a portion of the same sample to determine only vanadium, and thereby, both manganese and vanadium could be determined using the same sample.

Considering the values listed for manganese and vanadium in sea water, it was assumed that the long path method could be applied to the sample with no prior treatment other than the reduction of large amounts of any strong oxidants present. However, such a simple approach proved impossible. For some reason, color development in the sample solution was inhibited or suppressed and it was felt that some combination of components in the sample matrix must be the cause of the problem. It was further felt that the interference probably resulted from some organic species present in the sample, but no tests were performed to verify this. A separation via precipitation from alkaline medium with 8-hydroxyquinoline (oxine) was carried out. The precipitation brings down manganese and certain other metals (that act as trace scavengers) and render a clean sample, that is, one free of the organic matrix that is present in sea water. Although an enrichment was theoretically unnecessary, it was decided to effect one in order to increase the absorbance reading.

Three 25-ml portions of the sea water were taken through the procedure in a group. Each portion was made alkaline and the metals precipitated with oxine. The mixture was centrifuged, the supernatant liquid decanted, and the precipitate redissolved in acid without washing. The solution was brought to a volume of 10 ml in a volumetric flask with water. This resulting 10-ml sample preparation was then carried through the analysis. The results, reported as ng/ml manganese, from several repititions on the sample groupings, are presented in Table 5.

Table 5. Results of the Determination of Vanadium and Manganese (as Manganese) in Sea Water with o-Tolidine Using a 20-cm Cell

Sample	Manganese, ng/ml	
1	4.10, 4.00, 3.95	average: 4.02
		s_x: 0.076
2	4.25, 4.15, 4.00	average: 4.13
		s_x: 0.126
3	4.00, 3.85, 3.90	average: 3.92
		s_x: 0.076
4	3.75, 3.90, 3.90	average: 3.85
		s_x: 0.087
5	4.00, 4.05, 4.00	average: 4.02
		s_x: 0.029
standard deviation of the averages: 0.107		

Although the results obtained in this way are within the range to be expected for the amounts of manganese and vanadium reported for sea water, it was still not established whether they were the correct ones for the sample at hand. While the oxine separation had obviously removed the most offensive portion of the matrix, the possibility still existed that some interferences had been carried down with the sought for elements. Therefore, an artificial sea water was prepared; artificial with respect to the inorganic materials. The appropriate amounts and types of salts, with the exception of manganese and vanadium, were dissolved in water. Such water was then treated as the sea water "blank" and carried through the procedure. Its absorbance value was then compared with that obtained with a reagent blank. The two values differed by only 0.001 absorbance unit, which is definitely not significant. Thus, it could be concluded that the oxine separation had effectively removed the interferences. However, it still was not clear whether the result was the correct one.

Since methods that are capable of determining these two elements at such low levels, such as neutron activation analysis or carbon oven atomic absorption spectrometry, were unavailable during this research, the time proven method of spiking was used as a check. This technique involved the addition of a known amount of manganese to the sample, the analysis of the sample, and the examination of the results to see if the spiked amount was recovered.

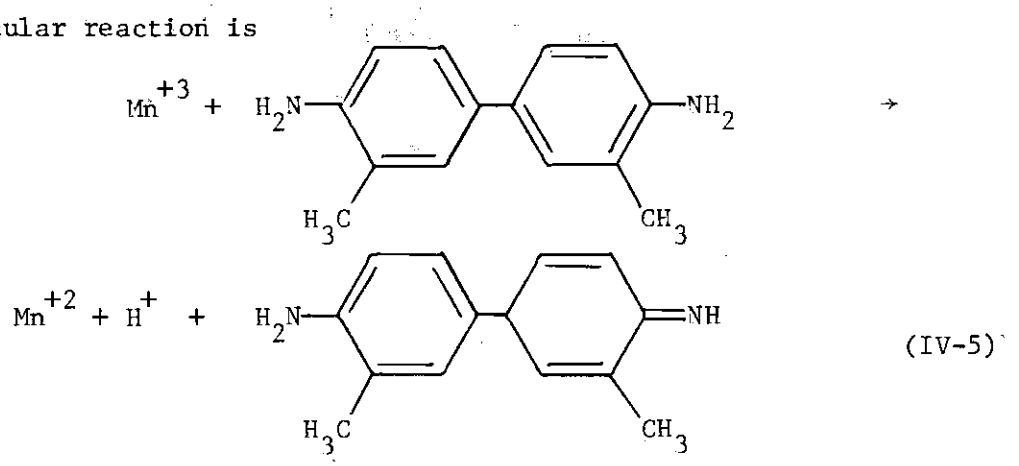
Manganese in the amounts of 1, 2, 5, and 10 ng/ml was added to sea water samples. The earlier determinations had established a value

of 4 ng/ml as manganese. The results of the spiked samples are given in Table 6. As can be seen, the method is able to find the spikes, with results that are in excellent statistical agreement for replicate determinations.

The recovery of the spiked amount per se is not yet proof that the procedure works reliably. However, in connection with the previously described correct results on solutions containing exactly known amounts of manganese, the spiking experiments can be taken as verification that the procedure is applicable to sea water or equivalent sample materials.

Section VI: Investigations into the Meriquinoid

As was mentioned earlier in this work, in several instances the blue colored meriquinoid species occurred, rather than the quinonediimine. The particular reaction is



(IV-5)

As can be seen, only one manganese(III) is required for the formation of the meriquinoid, whereas two are required for the formation of the

Table 6. Results of the Analysis of Spiked Sea Water Samples

manganese, ng		
added	recovered	Δ%
25.0	25.2	0.80
50.0	49.1	-1.80
125	123	-1.36
250	255	2.00

quinonediimine (see reaction (IV-1)). The meriquinoid was studied and found to have a molar absorptivity of 3.3×10^4 liters per mole-cm, slightly less than that of the quinonediimine. However, as one may recall, since the quinonediimine is formed in a reaction with two manganese ions, the apparent molar absorptivity is only 1.7×10^4. Because the meriquinoid is formed via the reaction of o-tolidine with only one manganese ion, its molar absorptivity with respect to manganese is still 3.3×10^4, or about twice that of the quinonediimine in this respect. In addition, there are LEDs available that emit at a wavelength at which the meriquinoid absorbs. Thus, the pulsed LED spectrophotometer described in Chapter III could be employed. With these thoughts in mind, an attempt was made to find the proper conditions for the determination of manganese via the meriquinoid of o-tolidine.

Several experiments showed that the meriquinoid was produced at higher pH values that the quinonediimine, hence the amount of sulfuric acid added to the solution was reduced accordingly. The meriquinoid formed more readily but was unstable, and eventually always was further oxidized to the quinonediimine. Several attempts were made to stabilize the meriquinoid, involving such approaches as raising the pH after the formation of the meriquinoid, addition of various redox buffers to create poised systems, and so on. Only very limited success was achieved.

Since the original purpose of this work was to develop methods that not only were more sensitive, but also more rugged, and easier to apply, the involved conditions necessary for even the rather

unsatisfactory use of the meriquinoid seemed to preclude further investigation here, and the meriquinoid studies were abandoned. Research is being conducted by Dobbs (33) into the general analytical potential of the meriquinoid.

Section VII: Other Experiments

There were several other avenues explored in the development of the method which did not bear fruit, but which warrant discussion. Many situations unique to trace analysis were encountered, posing problems not easily answered.

A great deal of experimentation, much of it unexpected, was required in the transition to the McKee-Pedersen instrument and long path micro cell. The major problem encountered was that of the introduction of the sample preparation into the microcell. Much work finally produced a satisfactory procedure, but further research was done to see if the process could be made easier. The addition of a surfactant to the sample preparation was tried, in hopes that this would ease the introduction process. Several surfactants were on hand in the research laboratory, and these were tried. Some of the surfactants inhibited the color development, making them worthless, others did not facilitate the sample introduction. There are, of course, many surfactants that were not tried, but which may be of help. It would be worthwhile to pursue research into this area, but lack of the necessary surfactants or funds to purchase them prevented such studies.

The problems concerning the color stability of the quinonediimine have been previously elaborated. After a satisfactory method of color stabilization had been developed, research by Dobbs (33) revealed a new approach. Dobbs found that recrystallization of o-tolidine yielded a purer reagent which gave a more stable color. The purified reagent has to be stored in a dessicator with sulfuric acid as the dessicant. Further research is being conducted in this area.

Section VIII: Conclusion

The desired objective of this portion of the investigation, namely the development of a more sensitive method for the determination of manganese, was attained through a combination of two of the three approaches listed in Chapter II. A chromogenic agent, o-tolidine, with a molar absorptivity over seven times that of permanganate, the colored species most often used in the photometric determination of manganese, was made to function as a selective reagent for manganese, with only vanadium posing any serious problems. A rather novel approach was used to avoid the vanadium interference when only small amounts of vanadium are present.

The other approach for increasing the sensitivity that was used here was the technique of long path photometry. The use of a 20-cm microcell greatly increases the sensitivity of the method.

The resulting analytical method meets the criteria of increased sensitivity, ease of applicability, and simplicity. The method was tested on a sea water sample and found to function properly.

CHAPTER V

CHEMICAL AMPLIFICATION REACTIONS

There exists another approach to increase sensitivity that has found growing attention in recent years, namely, chemical amplification. At the present time, the field is very fluid and it is, therefore, difficult to give a general definition of what is commonly called a chemical amplification reaction. The reader is referred to the review by Belcher (37) for a more detailed discussion.

Chemical amplification reactions received their first widespread application to analytical problems from chemists involved in organic microanalysis. Microanalysts were rather severely limited in the size of the sample available for the analysis, and faced great problems in the determination of a trace component. Therefore, they were forced to seek means to increase the sensitivity of the classical methods.

The obvious answer was to carry out a reaction or series of reactions that would, either directly or indirectly, increase the amount of the sought for substance or some species associated with it. This is distinctly different from an enrichment technique, which is usually understood to be a way to increase the concentration of the sought for species, commonly by reducing the solution volume in which that amount of the sought for species is contained. It is, of course, possible to combine the two techniques to gain an even greater increase in the sensitivity.

Amplification reactions, though not always termed thusly, have been known for over a century. The term "amplification reaction" came into general usage when such reactions were applied to the problems of microanalysis, which occurred in the middle part of this century. For a long period, only a few reactions were used, mainly in organic microanalysis. However, there has been an expanded interest in amplification reactions in the last decade, as analysts have realized that by the general application of such reactions in trace analysis it is possible to obtain both greater sensitivity and precision in the determination.

Perhaps the best known amplification reaction is the one used by Leipert in the determination of iodine in organic compounds. In the Leipert reaction, iodide is oxidized quantitatively to iodate by the addition of bromine water and shaking. The excess bromine is then destroyed, iodide added in excess, with the following reaction occurring

$$IO_3^- + 5I^- + 6H^+ \rightarrow 3I_2 + 3H_2O \qquad (V\text{-}1)$$

with the original amount of iodine being amplified six-fold. This application aroused great interest because prior to this, the microdetermination of iodine had been done gravimetrically, since titrimetric methods were generally unreliable due to the one-to-one equivalence of iodine with thiosulfate

$$I_2 + 2S_2O_3^{=} \rightarrow 2I^- + S_4O_6^{=} \qquad (V\text{-}2)$$

Various analysts, most notably Mohr (69), Winkler (44), and Hunter (54), had also used a similar reaction sequence prior to Leipert, but with either chlorine or hypochlorite as the oxidant. Bugarszky and Horvath (55) first used bromine in 1909, but ten years before the publication of the technique put forward by Leipert.

This sort of reaction is termed a direct amplification reaction, that is, the constituent sought is amplified directly and measured as such. Direct amplifications are rare.

Viebock and co-workers (56) applied the Leipert reaction to the determination of alkoxyl groups. Viebock initially converts the alkoxy group, e.g. CH_3O-, to the corresponding iodide, CH_3I, next decomposes the iodide, oxidizes the iodide to iodate, using bromine water as the oxidant, and then proceeds as in reaction (V-1). Such an application gives an amplification factor of six with respect to the alkoxy group

$$CH_3O- \rightarrow CH_3I \rightarrow IO_3^- \rightarrow 3I_2 \equiv 6CH_3O- \qquad (V-3)$$

This is an example of an indirect amplification reaction, that is, some species associated with the sought for species is amplified in quantity and measured. The majority of amplification methods known today are based on indirect processes.

The designation "indirect amplification" can be applied to a great number of techniques. Many gravimetric techniques, while not increasing the number of molecules or atoms of the sought for substance, are in effect a type of indirect amplification, in which the mass of the

substance weighed is far greater than the mass of the sought for species. There is also the well known method for the determination of many metals via 8-hydroxyquinoline (oxine). The metal is precipitated with oxine, the oxinate filtered and washed, then redissolved in acid. The now free oxine is brominated with a measured excess of bromine, and the excess bromine determined iodometrically. Although no species has been amplified, there is an increased equivalence brought about through the determination of the excess bromine.

Belcher (38) has termed such a process an equivalence enhancement reaction. This is a better term, since it can be applied to such diverse techniques as the amplifications obtained by Weisz and co-workers using the ring oven.

Flaschka and Yarbro (27) have developed a very interesting method for the determination of chromium which is in effect an equivalence enhancement reaction. Chromium is usually determined photometrically with diphenylcarbazide, in which one molecule of chromogenic reagent reacts with one chromium. Flaschka and Yarbro used o-tolidine as the chromogenic reagent, and in their method, each chromium reacts indirectly via a manganese oxidation with one and one-half molecules of o-tolidine, thereby giving an amplification factor of 1.5 with respect to chromium. The details of the process are to some extent explained in Chapter IV.

There are numerous amplification and equivalence enhancement reactions known today, and rather than elaborate at length on each one, the more important ones are summarized in Table 7.

Table 7. Amplification Reactions Used in Analytical Chemistry

Species Determined	Species Amplified/ Property Enhanced	Amplification Factor	Method of Analysis	Ref
Ag	Ag		Ring Oven	72
Alkoxyl groups	I	6	Titrimetric	56
Al	I(oxine)	30	Titrimetric	67
α-Amino Alcohols	I	18	Titrimetric	39
$AsO_4^{\equiv}$	I	12	Titrimetric	45
AsO_2^-	I	3	Titrimetric	41
	I	14	Titrimetric	45
B	I		Photometric	37
Bi	NH_3	2	Titrimetric	48
	I	35	Titrimetric	49
	SCN^-	48	Titrimetric	50
	SCN^-	228	Titrimetric	43
Br	Br	6	Photometric	53
	Br	12	Titrimetric	73
	I		Titrimetric	73
CH_3Br	Br		Photometric	59
CO_2	C	24	Titrimetric	64
Ca	C	20	Titrimetric	68
Cl^-	I	12	Titrimetric	37
Cu	Cu		Ring Oven	72
	Br(oxine)	8	Titrimetric	57
Cr	I	12	Titrimetric	46
	absorptivity	1.5	Photometric	27
$CrO_4^{=}$	$CrO_4^{=}$		Ring Oven	72

Table 7 (continued)

Species Determined	Species Amplified/ Property Enhanced	Amplification Factor	Method of Analysis	Ref
Fe	Fe		Ring Oven	72
Hydrazines	I	6	Titrimetric	40
I	I	6	Titrimetric	37
	I	6	Titrimetric	73
	I	24	Titrimetric	62
	I	36	Titrimetric	65
Mg	Br(oxine)	8	Titrimetric	61
	I(oxine)	30	Titrimetric	58
Mn	I	20	Titrimetric	42
Mo	Br(oxine)	96	Titrimetric	37
Ni	Br(oxine)	8	Titrimetric	57
NO_3^-	NO_3^-	37	Titrimetric	60
O	I	6	Titrimetric	85
P	Mo(oxine)	4	Titrimetric	71
	Mo(oxine)	12	Titrimetric	63
	Mass	140	Gravimetric	70
Pb	Pb		Ring Oven	72
Sb	I	18	Titrimetric	52
$SO_3^=$	I	6	Titrimetric	41
$SO_4^=$	I	6	Titrimetric	51
$S_2O_3^=$	I	24	Titrimetric	47
Zn	Zn		Ring Oven	72

In Table 7, the species determined is given in the first column, the species amplified or enhanced in column two. If there is a definite amplification factor, it is given in the appropriate column. The finish, i.e. photometric, titrimetric, etc., is also given.

If one examines the table, it is readily apparent that a great number of the amplifications involve that of iodine. Even though there are several procedures for the amplification of iodine, there seemed to be room for some simplifications and improvements and it was therefore decided to more closely examine the situation; this is done in Chapter VI.

CHAPTER VI

PHOTOMETRIC TITRATION OF IODINE AFTER CHEMICAL AMPLIFICATION

The determination of iodine has been well studied and several excellent reviews (73,74,75) on the subject exist. It is possible to determine iodine by any of several techniques; titrimetry, photometry, and amperometry are among the more common. Of these, titrimetric methods are by far the most frequently used, with several types of end-point indication possible. Perhaps the best known of all the methods for the determination of iodine is the titration with thiosulfate, using starch as the indicator.

The reaction between iodine and thiosulfate is known to work well at all levels of iodine. At very low levels of iodine, the amperometric titration is most often used. However, a photometric titration can also be used at these levels, and is an accepted technique. With this in mind, a photometric titration technique was chosen for study here.

Several amplification reactions of iodine are known (65,66). An example of a rather interesting one is the method developed by Spitzy and co-workers, involving a two-phase system. Iodine is formed by oxidation and extracted into carbon tetrachloride, then reextracted as hypoiodite into alkaline aqueous solution; then iodide is obtained upon acidification and is oxidized to iodate with bromine. Excess bromine is destroyed, the solution acidified, iodide added, and the amplification

achieved. The amplification factor is six, with a cyclic application possible.

The interesting portion of the method of Spitzy is the use of a two-phase system to obtain the iodine separated from most other materials present. There seemed to be a good possibility of improving the method by modifying it in the following way. Once the iodine is in the organic phase it is treated with bromine water directly rather than after reextraction. Upon shaking, bromine oxidizes the iodine to iodate which moves into the aqueous phase while the excess bromine transfers to the organic phase and is automatically removed with no reduction (e.g. by formic acid) necessary. To the aqueous phase iodide is added and the iodine formed may either be titrated or else it can be extracted and again subjected to the bromine water treatment and another cycle conducted. The reaction is

$$5Br_2 + I_2 + 6H_2O \rightarrow 10Br^- + 2IO_3^- + 12H^+ \qquad \text{(VI-1)}$$

In carrying out the amplification in this way, the number of extractions is reduced by one in each cycle. Also, the use of sodium hydroxide is eliminated. This is significant because of the difficulty of obtaining iodine-free sodium hydroxide. The amplification factor is the same as that of Spitzy, but the elimination of the reextraction step, coupled with the reduction in the blank by the elimination of the sodium hydroxide, should improve the sensitivity.

There is also a rather interesting fact to be considered in the use of a photometric titration. The starch iodine complex is blue in color, and its absorbance curve is shown in Figure 12. As can be seen, it has a rather broad peak in the range of 600 to 650 nm, with the maximum depending on the starch fraction used. Considering this, it was decided to use the optoelectronic photometer described in Chapter III. An orange-red LED, Hewlett-Packard 5082-4658, which emits at 635 nm was chosen for use here.

Development of the Method

It was realized that there were several points that needed to be investigated in the development of the method. The first of these to be examined was whether or not the reaction would occur as expected. A very simple experiment was performed to test this. A few milligrams of iodine were dissolved in about 100 ml chloroform. A 10-ml portion of this solution was shaken with an equal volume of bromine water. The aqueous phase was collected, formic acid added to destroy any remaining bromine and to acidify the solution, then a few milliliters of a freshly prepared iodide solution added. The solution was swirled to aid in mixing, starch indicator solution added, and the solution swirled again. Then the solution was titrated with thiosulfate. For some reason, however, the result did not give the value expected for a twelve-fold amplification but was rather low, approximating a factor of nine. But the results as a whole were quite encouraging for such a crude experiment and showed that the method did have a good probability of success.

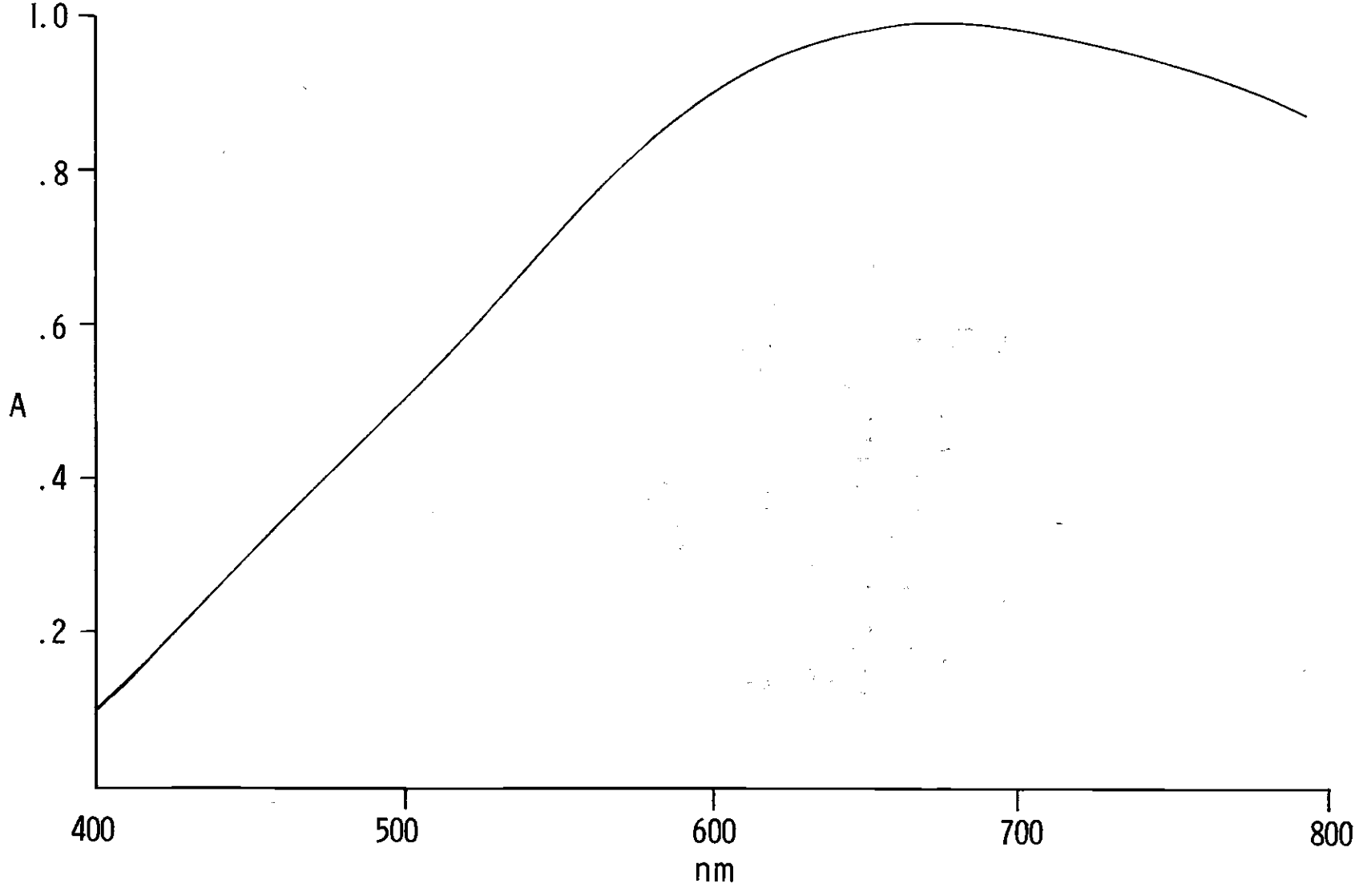


Figure 12. Absorbance Curve of Starch-Iodine Complex

Now efforts had to be directed toward the examination of the conditions necessary to achieve complete reaction. An initial thought was that perhaps the iodate formed was to some extent soluble in chloroform. A series of experiments were conducted to test this assumption. A solution was prepared from primary standard grade potassium iodate that had been dried in an oven at 110°C for three hours and stored in a dessicator over calcium chloride. Varying volumes of the 0.0005 F iodate solution was shaken with chloroform and bromine water. The aqueous phase was treated as before and the results are given in Table 8. As can be seen, there was no significant loss of iodate in the process. Therefore, the possible solubility of iodate in chloroform could be ruled out as a source of error.

The next point examined was the effect of shaking time on the amplification factor. The examination of this factor required a more quantitative approach than the earlier examination of the reaction. Therefore, the standard iodate solution that had been prepared was used for the in situ creation of iodine via reaction with an iodide solution in acidic medium. A check of the literature (75) had indicated that a two-stage extraction of the iodine formed into chloroform should be sufficient, so the extraction was performed with two 5-ml volumes of chloroform and the extracts combined. Care was taken to rinse the inside of the tip of the separatory funnel with distilled and deionized water after each extraction. The chloroform solution was then taken through the procedure. An equal volume of the standard iodate solution was treated with iodide and acid and titrated without amplification. The

Table 8. Recovery of Iodate After Shaking with Bromine Water and Chloroform

Iodate		
µg taken	µg found	Δ%
10.0	9.9	-1.00
20.0	20.0	0.00
30.0	30.5	1.67
40.0	39.8	-0.05
50.0	50.2	0.40
100.0	99.7	-0.30

volume of thiosulfate required for the titration of the unamplified sample should be one-twelfth of that required for the amplified one.

Shaking times were varied from one minute to thirty minutes. The results are presented in Table 9. As can be seen from this table, the recovery varies from 89% to 97%, with a maximum reached at the three-minute shaking time, and maintained through the ten-minute shaking time. From these results, it was decided to shake the system for five minutes.

The next factor investigated in the attempt to achieve complete recovery was the pH of the aqueous phase. This may seem unnecessary since the aqueous phase is saturated with bromine, but it did in fact prove important. With the small amounts of iodine present, it was not necessary to use just bromine water as the aqueous phase. Only a small volume of bromine water is required and to avoid having to work with only a one or two milliliter aqueous phase, water was added. It is known that at basic pH values, the iodine forms hypoiodite which will undergo a disproportionation into iodide and iodate. This is the system used by Spitzy. Therefore, it was necessary to see just how high a pH value could be tolerated. A series of buffers were prepared and used in the investigation of this point. Their pH values were 1, 3, 5, and 7. The results of the study are given in Table 10. As can be seen, the best results are obtained when using the pH 7 buffer, although the results with the use of the others are not that greatly different. However, this small difference allowed achievement of complete recovery, and the required pH is not greatly different from that of pure water, so no addition of a buffer solution was required.

Table 9. Effect of Shaking Time on Iodine Recovery After Amplification

Iodine µg taken	shaking time, min	µg found	% recovery
190.5	1	175.3	92
	2	180.9	95
	3	182.9	96
	5	183.1	96
	10	183.0	96
	20	171.4	90
	30	167.6	88
381.0	1	342.9	90
	2	350.5	92
	3	369.6	97
	5	370.0	97
	10	369.7	97
	20	342.8	90
	30	331.5	87
762.0	1	678.2	89
	2	685.8	90
	3	739.1	97
	5	738.9	97
	10	139.0	97
	20	670.5	88
	30	662.9	87

Table 10. Effect of pH of the Aqueous Phase on Iodine Recovery after Amplification

Iodine µg taken	µg found	pH of buffer	Δ%
762.0	701.1	1.00	-8.0
762.0	723.9	3.00	-5.0
762.0	739.1	5.00	-3.0
762.0	742.2	7.00	-2.6

It was also decided to check the supposition that no excess bromine would remain in the aqueous phase. This was done by shaking solutions of bromine water with chloroform and carrying the aqueous phase through the analysis. It was found that some bromine did remain in the aqueous phase. The possibility was considered of removing the bromine trace by a second or even third extraction. However, it was much simpler to add a few drops of formic acid to destroy the bromine with the added benefit of establishing at the same time the appropriate acidity.

Now that a general working method had been developed, the next step was to ensure that it is truly quantitative, reproducible, and to establish the sensitivity limites of the method.

Chemicals and Equipment

Chemicals

Distilled water, passed through a mixed-bed deionizer column was used exclusively. All common acid, base, and buffer solutions were prepared from reagent grade chemicals. The potassium iodide used was J.T. Baker "Analyzed Reagent" grade salt.

The organic chemicals and solvents were reagent grade or better, with the exception of the chloroform. The chloroform was a special spectral grade solvent, with very small ethanol content. The chloroform was further treated by shaking with water to remove the last traces of ethanol.

Spectrophotometer

The optoelectronic photometer described in Chapter III was used in this portion of the investigation. A Hewlett-Packard 5082-4658 LED

was used, which had a peak emission at 635 nm. The detector used was a Fairchild FPT-120 phototransistor.

Buret

The buret used was a No. 4834 Kimax (5-ml, 0.01 divisions) fitted with a platinum capillary tip.

Glassware

Common laboratory glassware such as beakers and flasks was used as needed. The titration vessel used was a 25-ml erlenmeyer flask. Class A volumetric glassware was used where needed and without further calibration.

Procedural Details

Reagents and Solutions

Potassium Iodide Solution, 0.1 F. Add about 2 g KI to 100 ml water. Slow oxidation of iodide to iodine can occur, so prepare the solution as needed.

Potassium Iodate Stock Solution. Add 0.01686 g KIO_3 to 100 ml water in a 2-liter flask. Shake until all solid is dissolved and then make to mark with water. A 1-ml aliquot of this solution, upon acidification and the addition of iodide, will yield exactly 30 µg iodine.

Sodium Thiosulfate Solution, 0.01 F. Add 1.58100 g $Na_2S_2O_3$ to 100 ml water in a 1-liter volumetric flask. Shake until all solid is dissolved and make to mark with water. Allow the solution to age for a day. A working titrant is prepared by a ten-fold dilution of this solution and is standardized against iodine generated from the iodate stock solution.

Bromine Water. Add three milliliters of bromine to about 25 ml water and stopper tightly.

Formic Acid. Concentrated reagent grade formic acid directly from the container.

Starch Solution. Add 3 g starch to about 3 ml boiled water, and make into a paste. Dissolve the paste in about 400 ml boiled water. The solution is stable for 1 or 2 days.

Sample Treatment

If all the analyte is present as iodine rather than iodide, all that is necessary is to extract it into chloroform. Two extractions with 5-ml portions of chloroform are necessary. If any iodide is present, it is necessary to oxidize it prior to the extraction. This can be done by the addition of one or two drops of a strong oxidant, such as permanganate or dichromate.

The volume of sample used in the extraction should be chosen so that the final sample preparation contains at least 1 µg/ml iodine after amplification.

Procedure

1. Place the 10 ml chloroform extract into a 60-ml separatory funnel. Add 5 ml water and 1 ml bromine water. Shake the funnel for five minutes.

2. Allow the two phases to separate and discard the chloroform. Withdraw the aqueous phase into a 50-ml erlenmeyer flask, and rinse the inside of the funnel tip. Add 5 ml formic acid, and mix well.

3. Add 5 ml of the iodide solution, mix well, and then add 2 ml of the starch indicator solution.

4. Align the flask in the light path of the photometer and titrate with thiosulfate solution.

5. Carry a 10-ml sample of pure chloroform through the procedure and titrate to determine the blank.

6. Plot the results of both titrations and determine the endpoint in the usual manner.

Results and Discussions

The only real question still open for discussion was how a set of sensitivity limits could be attained. It is possible to titrate a sample 1 μg/ml in iodine without amplification using the optoelectronic spectrophotometer, so iodine samples in the range of 100 ng/ml were carried through the amplification procedure and titrated.

Rather than standardize the thiosulfate against a primary standard, it was decided to standardize it against an iodine solution that was 5 μg/ml in iodate before acidification and addition of iodide.

Several titrations were performed and their average used as the thiosulfate titer. Then several samples originally 100 ng/ml in iodine were taken through the procedure. The results of the thiosulfate standardization are presented in Table 11, and the results of the 100 ng/ml samples are presented in Table 12. As can be seen in both cases, the results are in excellent agreement. The endpoint for the unamplified 1 μg/ml standard is 0.275 ml; the endpoint for the amplified 100 ng/ml standard is 0.331 ml, after the subtraction of the bromine blank. The theoretically calculated endpoint after amplification is

Table 11. Establishment of the Titer of a Thiosulfate Solution

µg iodine	volume thiosulfate, ml	titer, ml/µg x 10^{-2}
25.0	0.275	1.10
25.0	0.277	1.11
25.0	0.275	1.10
25.0	0.276	1.10
25.0	0.273	1.09
25.0	0.275	1.10
25.0	0.275	1.10
50.0	0.549	1.10
50.0	0.550	1.10
50.0	0.548	1.10
50.0	0.550	1.10
50.0	0.551	1.10
50.0	0.550	1.10
50.0	0.549	1.10
		average = 1.10
		s_x = 3.92 x 10^{-3}

0.330 ml. As can be seen from the results in Table 12, there is good agreement within the results.

Several solutions prepared from the same standard iodate solution were prepared by a second party and given to the experimentor. These solutions were used as unknown samples and carried through the procedure. The results are given in Table 13. The results are in very good agreement with the actual values, serving to show the validity of the method.

All the titration curves were calculated by using the raw transmittance values in a computer program. The program is given below.

```
 5: "INPUT THE MILLILITERS AND THE PPM OF THE IODINE SOLUTION
    USED"
 6: "MILLILITERS USED";INPUT Q/: "PPM OF THE SOLUTION";/INPUT P
10: DIM A(30)/DIM B(30)/DIM E(30)
11: C=O/M=O
15: "INITIAL VOLUME;/INPUT D
17: "INCREMENT":/INPUT F
20: "ML","%T"
30: B (C)=M/: B (C),/INPUT A(C)
35: M=M+F
40: IF A (C)=999 THEN 55 /C=C+1
50: GO TO 30
55: Z=A (O)
60: FOR X=1 TO C-1
62: A(X)=A(X)-Z
63: NEXT X
```

Table 12. Results of the Photometric Titration of a 100 ng/ml Iodine Standard (After Amplification)

ng iodine taken	ml thiosulfate used	theoretical titer
2500.0	0.332	0.330
2500.0	0.328	0.330
2500.0	0.330	0.330
2500.0	0.329	0.330
2500.0	0.330	0.330
	average = 0.331	

Table 13. Results of the Determination of Iodine

ng iodine taken	found	Δ%
100.0	97.2	2.8
150.0	148	1.3
200.0	202	1.0
300.0	296	1.3
500.0	506	1.2

```
 65:  FOR X=1 TO C-1
 67:  A(X)=A(X)/A (C-1)*100
 68:  E(X)=2=-LOG(A(X))/2.303
 69:  E(X)=E(X)*(D+B(X))/D
 70:  NEXT X
 75:
 76:  "RESULTS FOR THE TITRATION OF"Q"ML OF A"P"PPM STANDARD
      IODINE SOLUTION"
 80:  "ML","ABS","%T"
 85:  A(C)=0
 90:  FOR X=C TO C-1
100:  B(X),E(X),A(X)
110:  NEXT X
```

Conclusion

Because of the simplicity of the method and the freedom from interference, it was not necessary to do a tremendous amount of work in its development. It is simple, straightforward, and very easy to apply. A lower limit of 100 ng/ml iodine before amplification was easily attained. With the use of a microburet an even lower limit is possible. This high sensitivity, combined with the many analytical determinations that can be carried out iodometrically, make this method extremely useful.

CHAPTER VII

THE EFFECT OF NONMONOCHROMATIC LIGHT IN ANALYTICAL PHOTOMETRY

Spectrophotometric errors may arise because of the use of non-monochromatic light. A formal proof of the requirement of strictly monochromatic light has been presented by Loudon (76). Kortum (77) has discussed the consequences in the simple case that the incident light consists of only two components. Lothian (78), Strong (79), Wentworth (80), and Poulson (81) have presented quasi-mathematical treatments of various types of non-monochromaticity. Broderson (82) has presented an excellent paper on slit width effects on monochromaticity. Goldring and co-workers (83) gave some approximate calculations of the error for some cases where the intensity of the incident light is a triangular function of the wavelength. Asmus (84) has given a procedure which introduces less approximations than the one presented here, but for each problem a special calculation must be made and general conclusions cannot be drawn.

In trace photometric techniques, one generally tends to neglect any errors that might arise from non-monochromatic light, and in practice, such an assumption is usually valid. However, when using much longer cells and chromogenic agents with much higher molar absorbitivities, one does not always work in the range of the calibration curve in which nonmonochromacy can be tolerated. In the present investigation,

formulae are derived which may have a general applicability and results are given for the calculation of the photometric error in certain selected cases.

Derivation of Equations: Parabolic Curves

Assumptions

For simplicity the wavelength λ at the maximum (or minimum) absorption of the component measured (see Figure 13) is taken as reference and assigned the value zero. The absorbance at this value $\lambda = 0$ is A_o; at other wavelengths A_λ is often given in the first approximation by

$$A_\lambda = A_o(1-\beta\lambda^2) \qquad \text{(VII-1)}$$

where β is a measure of the sharpness of the absorption curve. β is positive for a maximum and negative for a minimum. For $\beta > 0$ the half-bandwidth is defined in the usual way as the wavelength region where A_λ varies between $\frac{1}{2}A_o$ and A_o. The value of the half-bandwidth is $\sqrt{2/\beta}$. For negative β values a half-bandwidth with physical significance cannot be defined in the usual way. Therefore, for the purpose of this discussion it is defined as the wavelength region where A_λ varies between A_o and $1\frac{1}{2}A_o$, because with this definition it has the same value of $\sqrt{2/\beta}$ as in the case for $\beta > 0$. Since A_λ cannot be negative zero-value is assigned to A_λ for values of β and λ where equation (VII-1) would suggest negative values for A_λ.

For the intensity of the light falling on the cell, I_λ^o (see Figure 14), the function

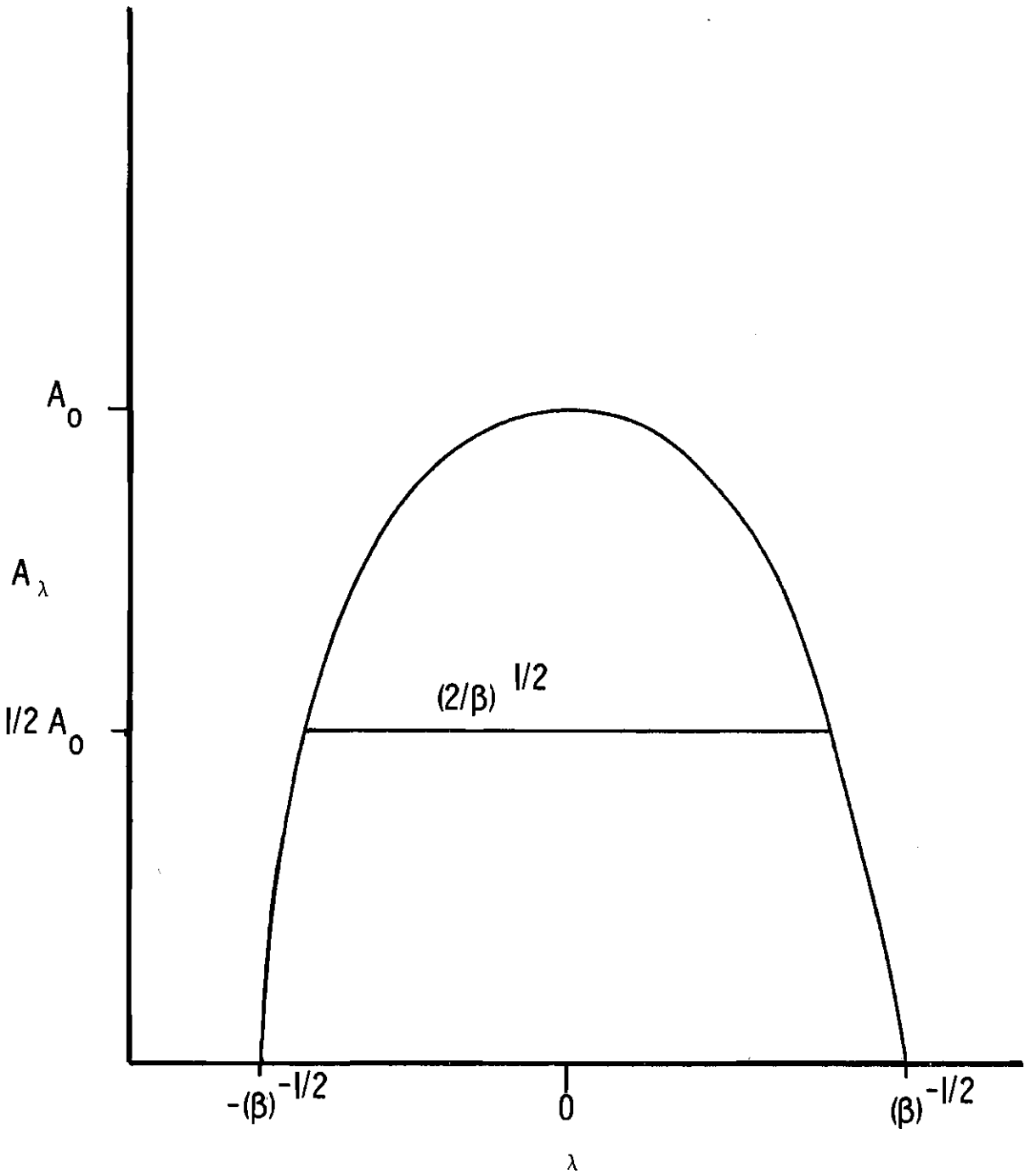


Figure 13. Absorbance as a Function of Wavelength, λ, for a Parabolic Curve

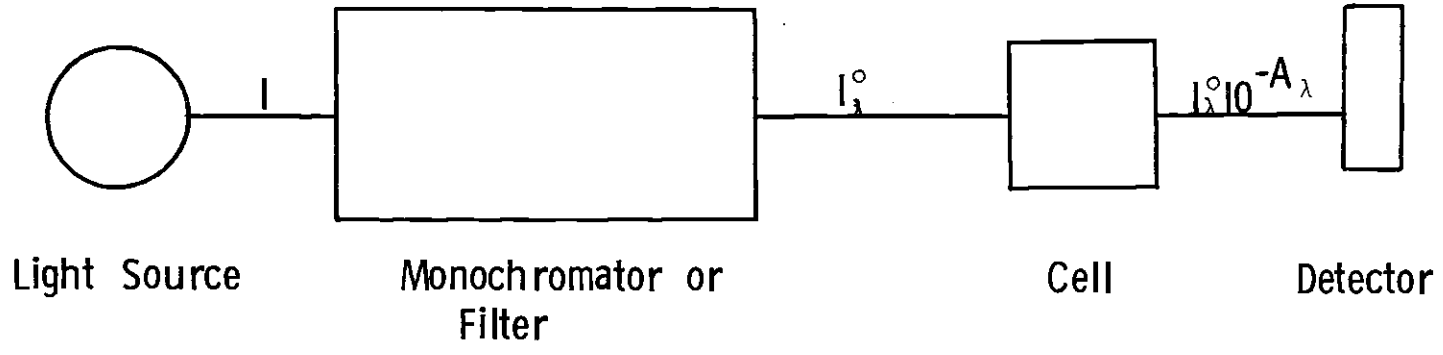


Figure 14. Light Intensity at Successive Places in the Spectrophotometer

$$I_\lambda^o = I^o[1 - \alpha(\lambda - z)^2] \qquad \text{(VII-2)}$$

is assumed, where I^o is the maximum intensity of the incident light falling on the cell; z is the difference in wavelength units between the maximum of the light intensity curve (see Figure 15) and the maximum or minimum of the absorption curve. α is always positive, the half-bandwidth is $\sqrt{2/\alpha}$ and $I_\lambda^o = 0$ at $\lambda = z \pm 1/\sqrt{d}$. Assuming that the spectrophotometer is not grossly non-monochromatic, the wavelength dependency of the light source intensity and the detector sensitivity can be ignored. If, however, there is gross non-monochromaticity or great variations in these two parameters, they will have to be considered. Rather than the simple single integration eventually arrived at here, a much more complicated triple integration would have to be used, and the solution of such an integral would require a large amount of time on a large and sophisticated computer.

If one ignores the influence of these factors, equation (VII-2) is in good agreement with the absorption curve of most filters, and prism and grating monochromators.

Losses of light intensity independent of the wavelength, λ, are unimportant. The presence of stray light, that is light of a greatly differing wavelength and much lower intensity, can be dealt with in a similar fashion. It would, however, require the construction of another set of overlap integrals. Since this problem is usually not encountered very much in modern photometry, it will not be considered here and the

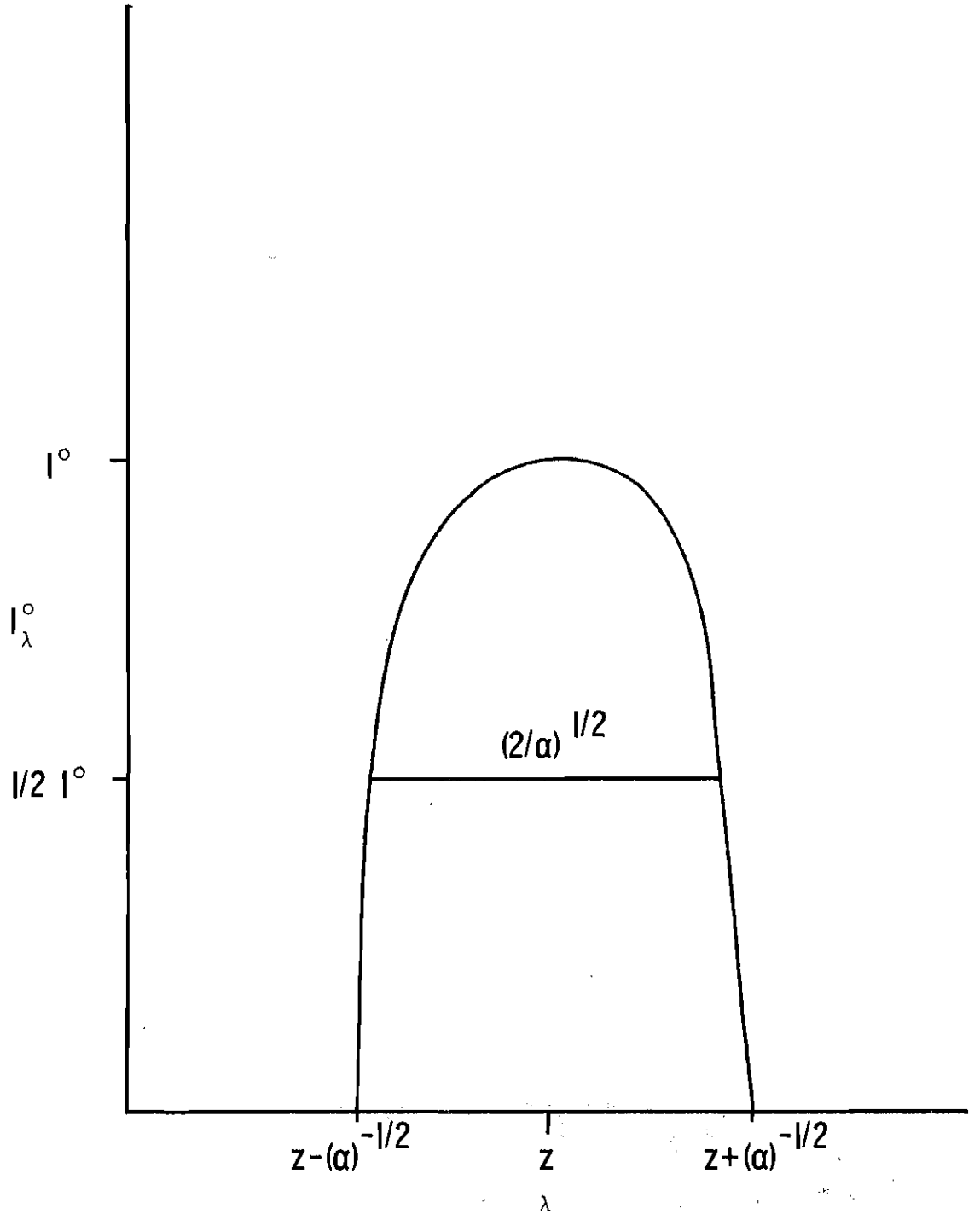


Figure 15. Intensity of Incident Light, I_λ°, as a Function of Wavelength, λ, for a Parabolic Curve

present discussion will be limited to the consideration of light distributed about a central wavelength.

Calculations

For calculations introduction of the quantities b, k, A, and B is useful

$$b = z\sqrt{\alpha}$$

$$k = \sqrt{\alpha/|\beta|}$$

$$E = 1 - \alpha(\lambda - b/\sqrt{\alpha})^2$$

$$B = 10^{-A_o(1 - \beta\lambda^2)}$$

The physical meaning of b is

$$b = \frac{\lambda_{Io} - \lambda_{Ao}}{\text{half-bandwidth of } I_\lambda^o \text{ curve}} \cdot \sqrt{2}$$

Calculations are made only for $b > o$. Symmetry considerations show that the same results are found for negative values of b.

The physical meaning of k is

$$k = \frac{\text{half-bandwidth of } E_\lambda \text{ curve}}{\text{half-bandwidth of } I_\lambda^o \text{ curve}}$$

The measured absorbance, A_m, is a mean value over the wavelength region considered, and may be given by the general formula

$$A_m = \log \frac{\int I_\lambda{}^o d_\lambda}{\int I_\lambda{}^o 10^{-A\lambda} d_\lambda} \qquad \text{(VII-3)}$$

The relative error is

$$\frac{\Delta A}{A_o} = \frac{A_m - A_o}{A_o} \qquad \text{(VII-4)}$$

Relative error is the term in common usage; it is more correctly the deviation from the maximum absorbance value caused by the use of non-monochromatic light. To analytical chemists, it is the non-linearity in the calibration curve.

Derivation of Equations: Gaussian Curves

The previous discussion was for the case where absorbance wavelength and light-intensity versus wavelength curves are both parabolic in nature. Here the case where both curves are Gaussian is considered. These two cases were chosen as being the most probable to encounter in actual work.

Assumptions

The same assumptions concerning wavelength designations are applied here as in the case for parabolic curves. However, the absorbance at some wavelength λ is given by

$$A_\lambda = A_o e^{-\frac{1}{2}(\lambda/\beta)^2} \qquad \text{(VII-5)}$$

The half-bandwidth is defined as $2\beta\sqrt{2\ln 2}$ (see Figure 16).

For the intensity of the light falling on the cell, I_λ^o, the equation

$$I_\lambda^o = I^o e^{-\frac{1}{2}((\lambda - z)/\alpha)^2} \qquad \text{(VII-6)}$$

is assumed, where I^o is the intensity of the incident light falling on the cell, z is the difference in wavelength units between the maximum of the light intensity curve and the maximum of the absorption curve. The light intensity curve is shown in Figure 17.

Similar assumptions have been made as in the case for parabolic functions.

Calculations

The quantities b and k are also used here. They have the same physical meaning as before, but the mathematical relationships between these quantities and z, α, and β are slightly different.

$$b = \frac{\lambda_{I^o} - \lambda_{A_o}}{\text{half-bandwidth of } I_\lambda^o \text{ curve}} \cdot \sqrt{2} = 2/2\alpha\sqrt{2\ln 2}$$

$$k = \frac{\text{half-bandwidth of } A_\lambda \text{ curve}}{\text{half-bandwidth of } I_\lambda^o \text{ curve}} = \beta/\alpha$$

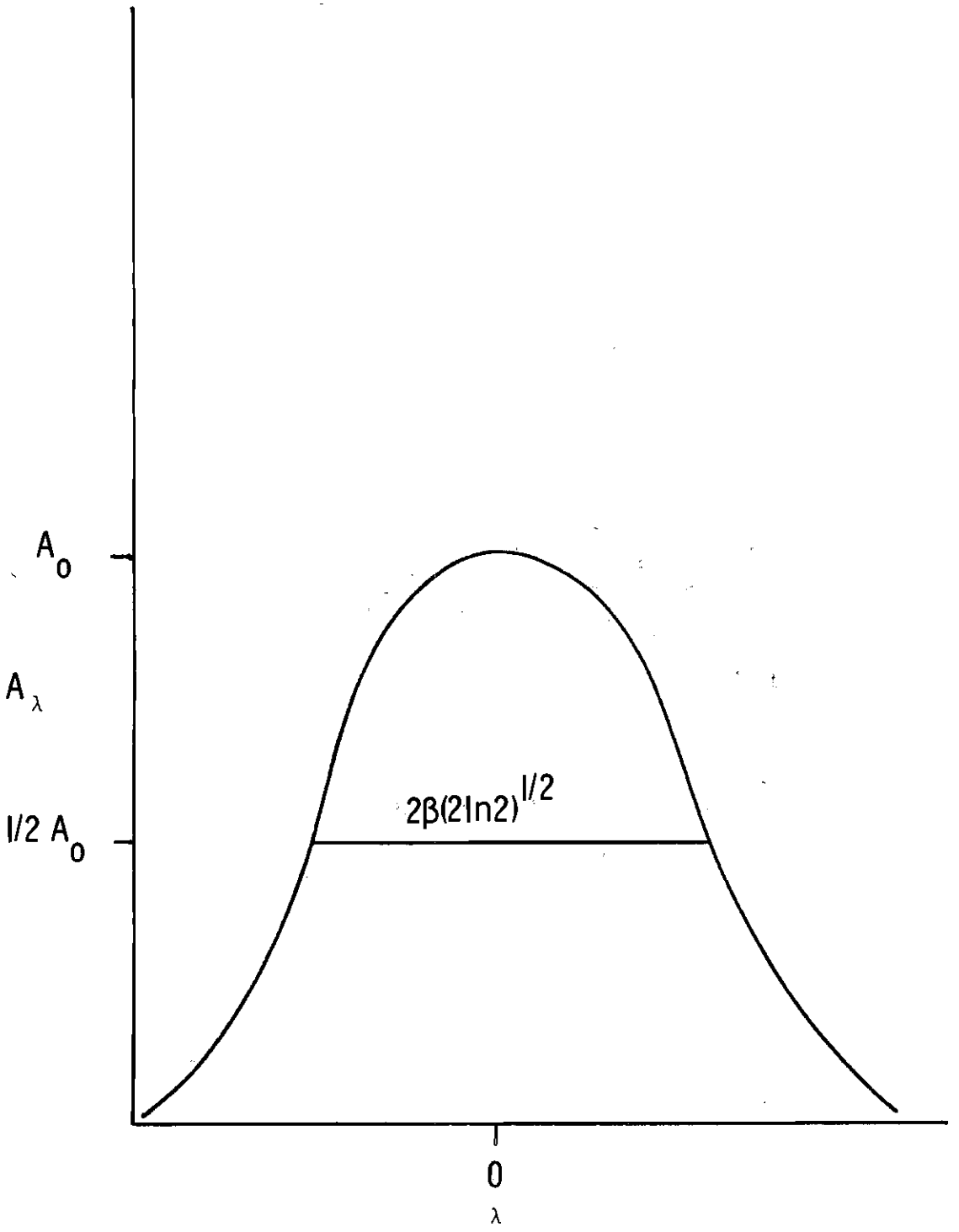


Figure 16. Absorbance as a Function of Wavelength, λ, for a Gaussian Curve

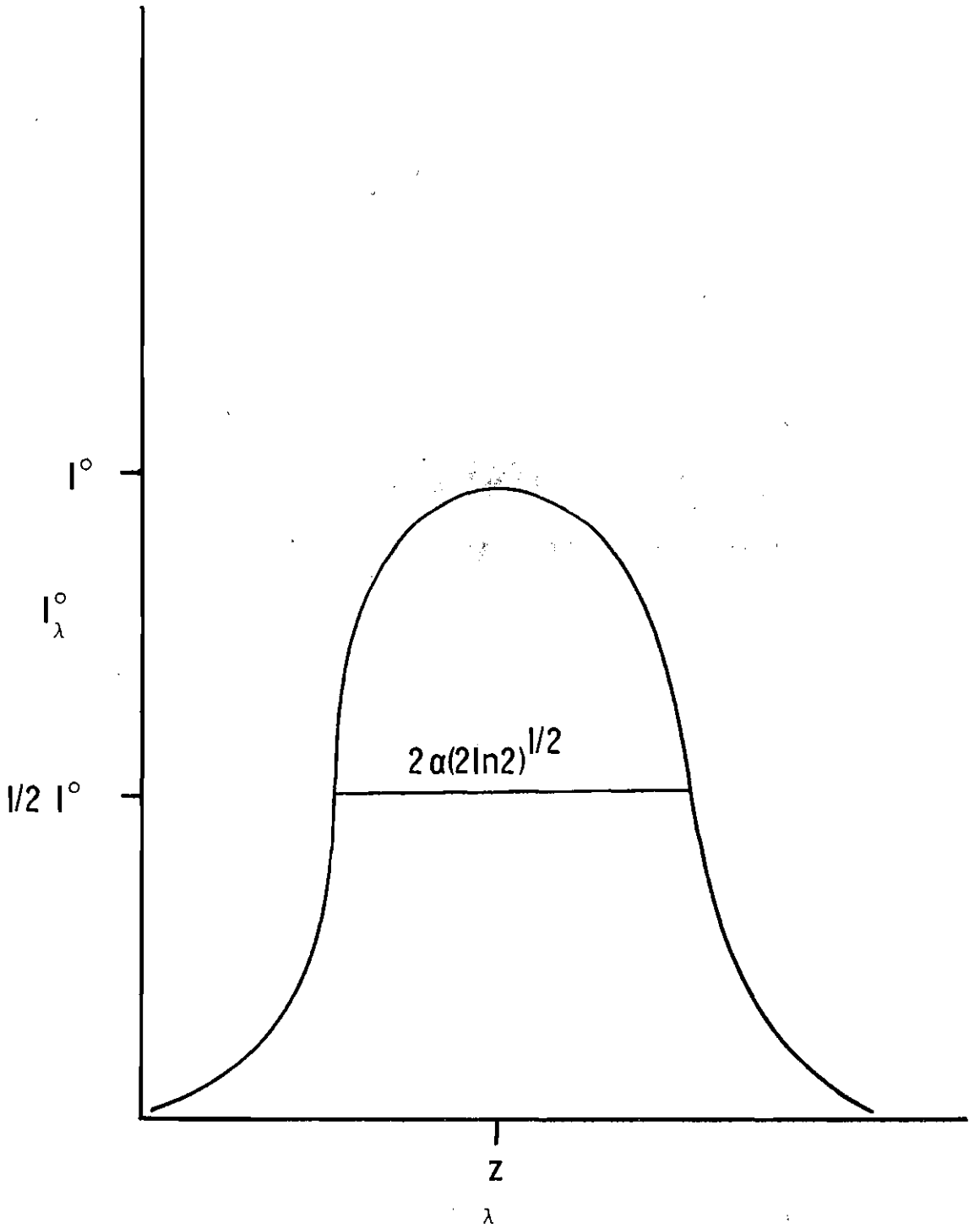


Figure 17. Intensity of Incident Light, I_{λ}°, as a Function of Wavelength, λ, for a Gaussian Curve

The measured absorbance A_m and the relative error are given by the same formulae as before.

Results

Both parabolic and Gaussian functions were considered. The appropriate equations were programmed into a PDP-8E computer using BASIC.

The only cases considered were for $\beta > 0$. The results are presented graphically in Figures 18 and 19.

Figure 18 shows the percent error as a function of the overlap of the absorbance curve and the intensity curve for parabolic functions. Varying k values are considered.

Figure 19 shows the same curves for Gaussian functions.

Examination of the figures shows that individual values of α and β are unimportant, but that the quotient $\alpha/|\beta|$ is the influential parameter. The curves show that in some cases the relative error is practically independent of the absorbance, a very favorable situation for calibration curves. In other cases, the relative error is nearly a linear function of absorbance, while in a very few cases the relationship is of a more complicated nature. In analytical photometry, the curves may often be used to predict approximately the deviation from linearity of the calibration curve if the wavelength dependence of the light absorption of the compound measured is known.

In the case of Gaussian functions, the relative error almost always is linearly increasing with increasing concentration.

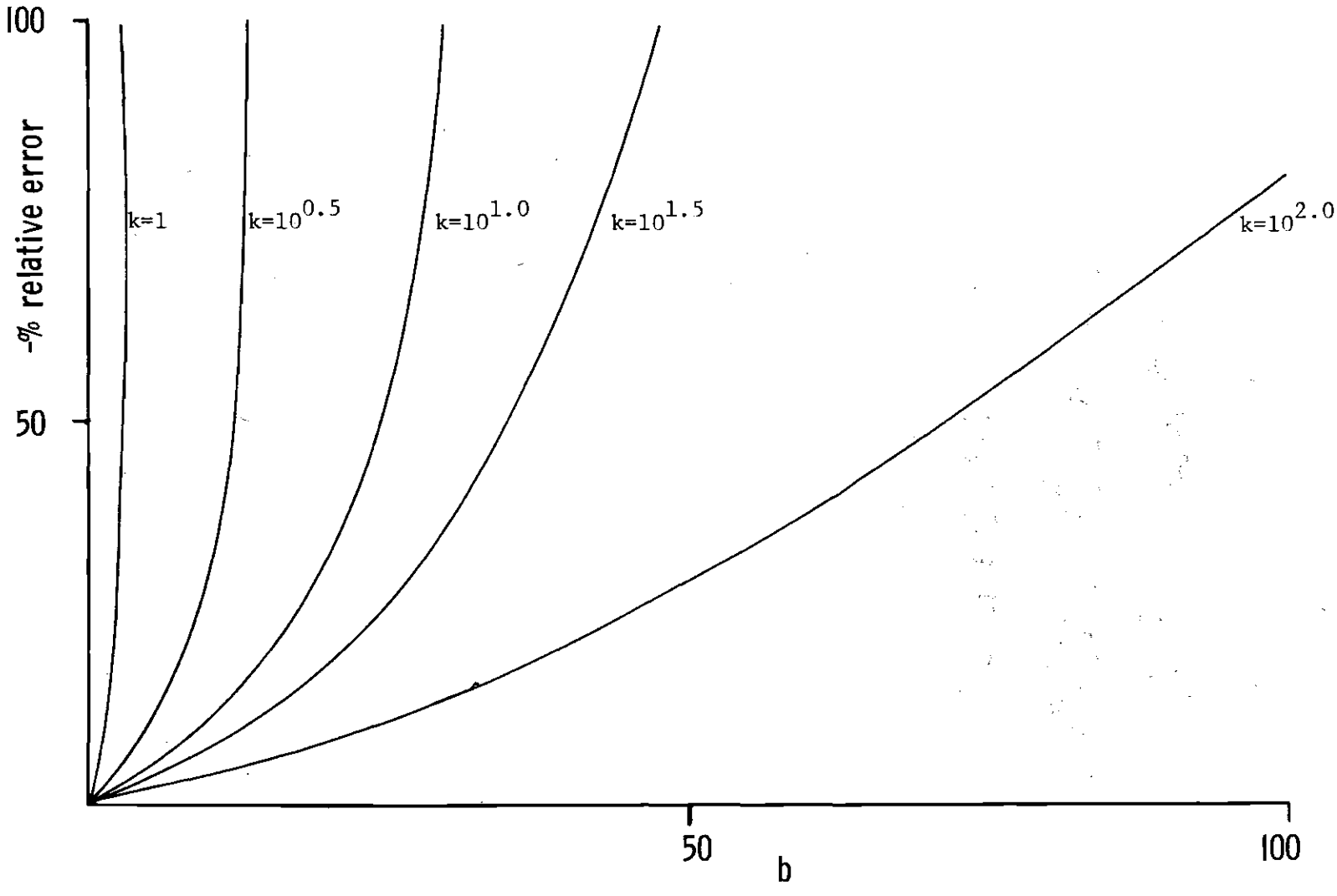


Figure 18. Percent Relative Error as a Function of Curve Overlaps for Parabolic Curves

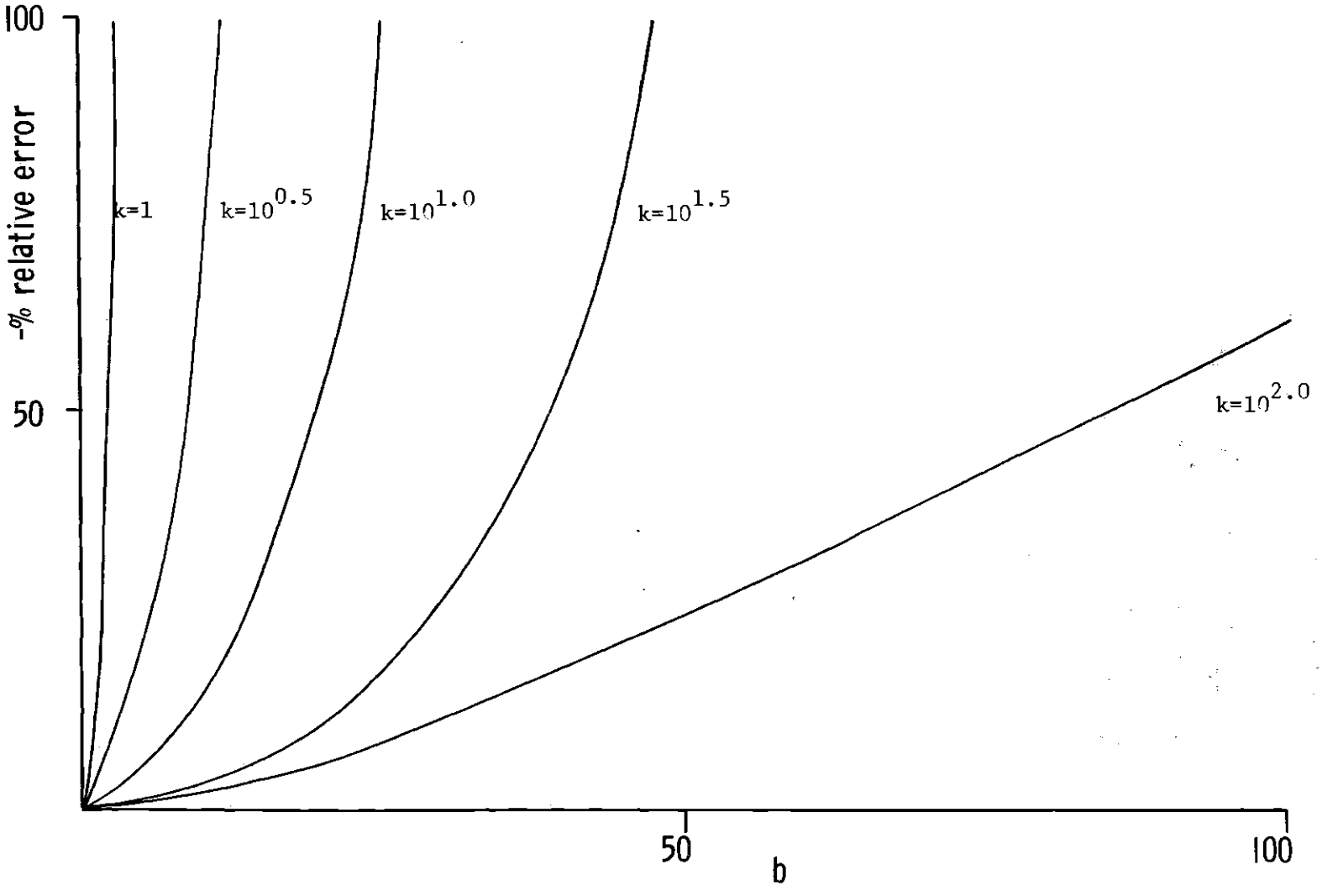


Figure 19. Percent Relative Error as a Function of Curve Overlaps for Gaussian Curves

REFERENCES

1. H. Flaschka, Pure and Applied Chem., 25, 1 (1971).

2. I.H. Tipton, The Distribution of Trace Metals in the Human Body, M.J. Inseren, editor, J.B. Lippincott Co., Philadelphia, 1960, pp. 201-225.

3. Anon., Chemical and Engineering News, 56, 6 (1978).

4. H. Belcher and D. Betteridge, Talanta, 12, 129 (1965).

5. C. Cheng, Anal. Chem., 33, 783 (1961).

6. A.L. Wilson, Talanta, 17, 21 (1970).

7. L. Pszonicki, ibid., 24, 613 (1977).

8. K. Vierordt, Pogg. Ann., 151, 119 (1874).

9. H. Flaschka and R. Barnes, Anal. Chim. Acta, 63, 489 (1973).

10. H. Flaschka and J.J. Tice, IV, Talanta, 20, 423 (1973).

11. E.A. Braude, Determination of Organic Structures by Physical Methods, Volume 1, E.A. Braude and F.C. Nached, editors, Academic Press, New York, 1955, p. 131.

12. R.L. Barnes, Increased Sensitivity in Photometric Determinations; Ph.D. Thesis, Georgia Institute of Technology, Atlanta, Georgia, June, 1972, pp. 18-21.

13. D.C. Paschal, Increased Sensitivity in Extraction-Photometric and Flame Photometric Determinations; Ph.D. Thesis, Georgia Institute of Technology, Atlanta, Georgia, June, 1974, pp. 24-26.

14. H. Flaschka and R. Barnes, Microchem. J., 17, 588 (1972).

15. H. Flaschka, C. McKeithan and D. Paschal, Microchem. J., 18, 152 (1973).

16. M.L. Coulter and H.A. Flaschka, Microchem. J., 21, 411 (1976).

17. M.A. Leonard, Comprehensive Analytical Chemistry, Volume VIII, G. Svehla, editor, Elsevier Scientific Publishing Co., New York, 1977, pp. 207-389.

18. H. Flaschka, C. McKeithan and R. Barnes, Anal. Lett., 6, 585 (1973).

19. G.E. O'Brien, J.V. Hornstein and H.A. Flaschka, Microchem. J., 22, 548 (1977).

20. H. Flaschka and M.L. Coulter, Essays on Analytical Chemistry, E. Wänninen, editor, Pergamon Press, New York, 1977, pp. 253-277.

21. H.A. Laitinen and W.E. Harris, Chemical Analysis, Second Edition, McGraw-Hill, Inc., New York, 1975, p. 330.

22. H.H. Willard and L.H. Greathouse, J. Amer. Chem. Soc., 60, 2869 (1938).

23. S. Bruno, Accad. pugliese sci., Atti e relaz., 11, 409 (1953).

24. J. Barek and A. Berka, Anal. Lett., 8, 57 (1975).

25. J. Maly and H. Fadrus, Analyst, 99, 128 (1974).

26. H.A. Flaschka, S. McClure and J.V. Hornstein, work in print.

27. S.K. Yarbro, Two Photometric Methods for the Determination of Chromium in Biological Material; Ph.D. Thesis, Georgia Institute of Technology, Atlanta, Georgia, June, 1976, pp. 53-64.

28. H. Flaschka and S. Yarbro, unpublished work.

29. J.J. Lingane, Analytical Chemistry of Selected Metallic Elements, Reinhold Publishing Co., New York, 1966, p. 75.

30. M.D. Cooper and P.K. Winter, Treatise on Analytical Chemistry, Volume VII, Part II, I.M. Kolthoff and P.J. Elving, editors, John Wiley and Sons, Inc., New York, 1967, p. 467.

31. F. Feigl, Spot Tests, Volume I, Elsevier Publishing Co., New York, 1954, p. 459.

32. J. Stary, The Solvent Extraction of Metal Chelates, MacMillan Co., New York, 1964, p. 119.

33. C. Dobbs, work in progress.

34. A.W. Morris, Anal. Chim. Acta, 42, 397 (1968).

35. L.R. Robinson, R.A. Dixon and E.D. Breland, Water Sewage Works, 115, 80 (1968).

36. H.H. Willard, L.L. Merrit and J.A. Dean, Instrumental Methods of Analysis, Fifth Edition, Van Nostrand Co., New York, 1974, pp. 42-81.

37. R. Belcher, Talanta, 15, 357 (1968).

38. Idem., ibid., 24, 533 (1977).

39. A. Besada and Y.A. Gawargious, ibid., 21, 1247 (1974).

40. Y.A. Gawargious and A. Besada, ibid., 22, 757 (1975).

41. A. Besada, Y.A. Gawargious and S.Y. Kareem, ibid., 23, 392 (1976).

42. J.W. Hamya and A. Townshend, ibid., 19, 141 (1972).

43. R. Belcher, S. Liawruangrath and A. Townshend, ibid., 24, 590 (1977).

44. L.W. Winkler, Z. Anal. Chem., 39, 85 (1900).

45. S.K. Tobia, Y.A. Gawargious and M.F. El-Shahat, ibid., 265, 23 (1973).

46. A. Besada, ibid., 271, 368 (1974).

47. S.K. Tobia, Y.A. Gawargious, A. Besada and S.Y. Kareem, ibid., 277, 376 (1975).

48. C. Mahr, ibid., 93, 433 (1933).

49. V.J. Straub, ibid., 76, 108 (1929).

50. J. Stamm and M. Goehring, ibid., 115, 1 (1935).

51. R. Wildenstein, ibid., 1, 323 (1862).

52. Y.A. Gawargious, L.S. Boulos and A. Besada, Analyst, 101, 458 (1976).

53. G. Hunter and A.A. Goldspink, ibid., 79, 467 (1954).

54. A. Hunter, J. Biol. Chem., 7, 336 (1909).

55. S. Bugarszky and B. Horvath, Z. Anorg. Allgem. Chem., 63, 184 (1909).

56. F. Viebock and C. Brecher, Ber., 63, 3207 (1930).

57. N.H. Furman and J.F. Flagg, Ind. Eng. Chem., Anal. Ed., 12, 738 (1940).

58. J.P. Nielsen, ibid., 11, 1649 (1939).

59. V.A. Stenger, S.A. Shrader and A.W. Beshgetoor, ibid., 11, 121 (1939).

60. G. Szabo and L.G. Bartha, Nature, 166, 309 (1950).

61. R. Berg, Pharm. Ztg., 71, 1542 (1926).

62. R. Belcher, J.W. Hamya and A. Townshend, Anal. Chim. Acta, 49, 570 (1970).

63. R. Belcher and P.C. Uden, ibid., 42, 180 (1968).

64. W. Schöniger, Microchem. J., 11, 469 (1966).

65. H. Spitzy, H. Skrube and F.S. Sadek, Mikrochim. Acta, 375 (1953).

66. U. Fritsche, ibid., 1322 (1969).

67. J.P. Mehling and C.J. Bernbach, Chem. Anal., 32, 80 (1943).

68. D.D. Van Slyke and F.J. Kreysa, J. Biol. Chem., 142, 765 (1942).

69. F. Szabadvary, The History of Analytical Chemistry, Pergamon Press, Oxford, 1963, p. 249.

70. F. Ibbotson and L. Aitchison, The Analysis of Non-Ferrous Alloys, Longmans, London, 1915, p. 183.

71. G.E.F. Lundell, J.I. Hoffman and H.A. Bright, Chemical Analysis of Iron and Steel, Chapman Hall, London, 1931.

72. H. Weisz and C. Tellegman, Mikrochim. Ichnoanal. Acta, 2, 258 (1965); C.A. 63:237if.

73. I.M. Kolthoff and R. Belcher, Volumetric Analysis, Volume III, Interscience, New York, 1957, pp. 199-350.

74. M.R.F. Ashworth, Titrimetric Organic Analysis, Parts I and II, Interscience, New York, 1964.

75. I.M. Kolthoff, E.B. Sandell, E.J. Meehan and S. Bruckenstein, Quantitative Chemical Analysis, Fourth Edition, MacMillan Co., London, 1969.

76. G.M. Loudon, J. Chem. Ed., 41, 391 (1964).

77. G. Kortüm, Kolorimetrie, Photometrie, und Spektrometrie, Springer-Verlag, Berlin, 1962, pp. 41-47.

78. G.F. Lothian, Analyst, 88, 678 (1963).

79. F.C. Strong, Anal. Chem., 24, 338 (1952).

80. W.E. Wentworth, J. Chem. Ed., 43, 262 (1966).

81. R.E. Poulson, Applied Optics, 3, 99 (1964).

82. S. Brodersen, J. Op. Soc. Amer., 44, 22 (1954).

83. L.S. Goldring, R.C. Hawes, G.H. Hare, A.O. Beckman and M.E. Stickney, Anal. Chem., 25, 869 (1953).

84. E. Asmus, Optik, 9, 108 (1952).

85. J. Unterzaucher, Ber., 73B, 391 (1940).

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